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NITROGEN IN PROTEIN BODIES.

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To properly differentiate and classify the protein bodies it is necessary to employ some method based on the structure of their molecules. Many of the color reactions, it is true, give us evidence of certain complexes in the protein molecule, but most of these reactions are characteristic of the protein bodies in general, and in but few cases distinguish between individual proteins. Furthermore, these reactions give no quantitative measure of the different complexes which cause them, and in these quantitative relations lies one of the most important differences between the several protein bodies. Until recently, knowledge regarding the structure of the protein molecule has been chiefly obtained by detailed study of the decomposition products resulting from boiling the protein with strong acids. A quantitative determination is possible for only a few of these decomposition products and, as large quantities of pure material and much time and skill are required for the examination, investigations of this kind have been applied to only a few of the known proteins.

Kossel and Kutscher[1] have recently perfected their method for

[1] *Ztschr. physiol. Chem.*, **31**, 165 (1900).

determining the basic bodies, histidin, arginin and lysin, three of the most important decomposition products yet obtained from proteins. From their results, it would appear that one of the best means at present available for distinguishing between the several proteins lies in a determination of the proportion of these bases which they contain. We have accordingly tried to determine the proportion of the different classes of nitrogenous decomposition products yielded by several of the proteins previously prepared and studied in this laboratory but as, in many cases, only small amounts were available, it is not at present possible to apply Kossel and Kutscher's method to them all.

As preliminary to a more exact study of our preparations, we have used a method proposed by Hausmann[1] for determining the proportion of nitrogen in different forms of combination that occur among the decomposition products which result after boiling the protein for a long time with acids. Although this method has been subjected to very severe adverse criticism, we have found that, under suitable conditions, it yields fairly uniform results and affords a rapid means for approximately determining the relations of different preparations to one another. Thus we have found by its use that some of our preparations from different seeds which were so nearly alike in composition and reactions that no difference could be detected between them sufficient to warrant the conclusion that they were not the same chemical individual, yield such different proportions of nitrogen in the several forms of binding that there can be no longer any doubt that they are distinctly different substances. On the other hand, many preparations of different origin which we have heretofore considered to be identical, have yielded the same proportion of the different forms of nitrogen and consequently our former opinion respecting the identity of these protein preparations is very greatly strengthened. We do not assert that Hausmann's method is capable of yielding accurate results respecting the true proportion of nitrogen in the various forms of binding, but that it yields valuable comparative results under the conditions which we have employed, is evident from an examination of our determinations given in the following pages.

Hausmann's method consists in boiling the protein with strong hydrochloric acid until it is completely decomposed and then de-

[1] *Ztschr. physiol. Chem.*, **27**, 92 (1899).

termining: (1) The nitrogen which can be expelled from the solution as ammonia by distillation with magnesia; (2) that which is precipitated by phosphotungstic acid from the solution thus freed from ammonia; and (3) the nitrogen remaining in the filtrate from the phosphotungstic acid precipitate. In this way Hausmann thought the amide nitrogen (1), the diamino-nitrogen (2), and the remaining nitrogen (3), belonging almost wholly to monamino acids, might be quantitatively determined. This method has, however, received, such severe criticism that it has found but little application and the results obtained by it have been accorded but slight consideration.[1]

Hausmann supposed that the amount of amide nitrogen was quite accurately determined by his method and was characteristic for each proteid, but Henderson[2] soon showed that the amount of ammonia obtained depended on the strength of the acid used in decomposing the proteid and on the time of boiling.

Schulze and Winterstein[3] found that after precipitating the solution of the decomposition products of proteins with phosphotungstic acid, a small amount of ammonia could be obtained from the filtered solution by distilling with magnesia. In agreement with this observation, Hart[4] found that a somewhat greater proportion of ammonia was obtained by distilling the solution of the decomposition products with magnesia than with barium carbonate. From these facts it would appear that distillation with magnesia does not give an accurate measure of the amide nitrogen yielded by the protein.

Kutscher[5] next stated that, according to experiments by Gulewitsch, the phosphotungstic acid compound of arginine is notably soluble in water; that the phosphotungstic acid precipitate of the diamino acids is soluble in a sufficient excess of the precipitant; that in the mixture of acids obtained by boiling the protein with hydrochloric acid an immediate precipitate results on adding phosphotungstic acid, but that, on increasing the amount of the latter, a further precipitate appears after a time and that

[1] Hofmeister ("Ergebnisse der Physiologie," Vol. I, p. 778 (1902)), in whose laboratory Hausmann's work was done, says that more accurate estimations by Hausmann's process will soon be published by Th. Gümbel, and the objections raised against this method by Kutscher and others will be met.

[2] *Ztschr. physiol. Chem.*, **29**, 47 (1900).

[3] *Ibid.*, **33**, 563 (1901).

[4] *Ibid.*, **33**, 347 (1901).

[5] *Ibid.*, **31**, 215 (1900).

this time gradually becomes longer after each addition of the acid. This latter precipitate is crystalline, is soluble in water, dilute acids, and in acidified dilute phosphotungstic acid solution, and does not contain diamino acids.

Kutscher conducted experiments with Hausmann's method in which he employed different volumes and proportions of phosphotungstic acid for precipitating the diamino acids obtained from casein, the results of which, under the different conditions employed, varied materially from one another and very greatly from that obtained by Hausmann with the same protein. Nevertheless, two results, secured under the same conditions, agreed very closely. Kutscher concluded that Hausmann's method cannot yield useful results.

Wetzel[1] employed Hausmann's method with gelatin, conchiolin and silk glutin. He found the same amount of nitrogen, as ammonia, in gelatin as did Hausmann, but only one-half as much diamino nitrogen. He explains the difference by saying "The amount of nitrogen which is obtained is too low for dilute solutions, too high for very concentrated, since in the one case all the bases are not precipitated and in the other, besides the bases, probably amido acids are also precipitated."

Chittenden and Eustis[2] state that the amount of bases precipitated by phosphotungstic acid from solutions containing the decomposition products of the proteins is variable and cannot be taken as a quantitative measure of the same.

Schulze and Winterstein[3] next determined the amount of histidine, arginine and lysine which they were able to isolate from the decomposition products of several crude preparations of vegetable proteins, and found that the amount of nitrogen contained in these bases fell considerably below that precipitated by phosphotungstic acid, according to Hausmann's method. From this they conclude that phosphotungstic acid precipitates other decomposition products than the three determined.

Schulze and Winterstein[4] next studied the deportment of some monamino acids towards phosphotungstic acid and found that neither glycocol, optically active and inactive leucine, aminovalerianic acid or tyrosine were precipitated from their 5 per cent.

[1] *Ztschr. physiol Chem.*, **29**, 405 (1900).
[2] *Am. J. Physiol.*, **3**, 31 (1900).
[3] *Ztschr. physiol. Chem.*, **33**, 547 (1901).
[4] *Ibid.*, **33**, 574 (1901).

solutions by phosphotungstic acid, but that of phenylalanine under these conditions gave an oily precipitate which, after a time, became crystalline. About 50 cc. of cold water were required to dissolve the precipitate produced by 0.1 gram of this substance.

Whether phenylalanine is thrown down by phosphotungstic acid from solutions containing the decomposition products of proteins, depends on the concentration of the solution. Since this body has as yet been found only in small quantity among protein decomposition products, it may escape precipitation, but the known tendency of substances to separate together from solution may cause it to precipitate to a greater or less extent.

Although a careful examination of the phosphotungstic acid precipitates failed to reveal the presence of monamino acids, even when methods were used which would surely have shown the presence of even small amounts of leucine and tyrosine, they conclude that it is probable that monamino acids are, in fact, carried down with the phosphotungstic acid precipitate.

From the preceding statements, it is evident that Hausmann's method, as he defined it, cannot be used to determine accurately the proportion of amido-, diamino- and monamino-nitrogen in the decomposition products of the various proteins. It is possible, however, under suitable conditions, to obtain valuable *comparative* results by its use, whereby differences between the various proteins are made plainly evident.

Henderson's statement that the amount of ammonia obtained depends on the strength of acid with which the proteid is boiled, as well as on the time of boiling, is doubtless correct, but an examination of his determinations, made under different conditions, shows that it is necessary to much increase the strength of the acid or greatly prolong the boiling in order to materially affect the result. It is therefore possible, under similar conditions, to obtain a uniform proportion of ammonia by distilling with magnesia. That *all* the ammonia, thus obtained, is derived from ammonium salts formed by the action of the boiling acid on amides is improbable, in view of Schulze and Winterstein's observation and also that of Hart.

We have, in the following investigation, determined the ammonia yielded by various preparations of the same protein on distillation with magnesia, and have, as inspection of the figures given in the following pages shows, obtained such uniform results that

they afford a ready means for comparing supposedly identical proteins.

Whether Hausmann's method can be modified so that the results will show the true proportion of basic nitrogen yielded by the proteins, will require more extended investigations than have as yet been undertaken. It will be shown later in this paper that, under the conditions which we have employed, the amount of basic nitrogen precipitated by phosphotungstic acid corresponds pretty closely with that contained in the histidine, arginine and lysine which Kossel and Kutscher[1] found in several proteins from cereal grains, but falling, as would be expected from the known slight solubility of arginine phosphotungstate, a little below them.

Unfortunately, these investigators did not examine any of the proteins yielding large amounts of basic nitrogen, except histone, and we have at present no accurate knowledge of the proportion of arginine, histidine and lysine which any of these contain. The determinations made by Schulze and Winterstein show that edestin and conglutin contain large proportions of these diamino-acids, but they did not employ pure conglutin and their determinations were made by Kossel's older method which, supposedly, does not yield such reliable results as the later one, recently devised by Kossel and Kutscher. Furthermore, we have no evidence that these three diamino acids are the only basic decomposition products of proteins and it may well be that further investigation will reveal the presence of others in some of the vegetable proteins, especially those rich in nitrogen.

Kutscher lays much stress on the solubility of the phosphotungstic acid precipitate, but our experience has not led us to consider that it is soluble to such an extent as he would have us believe. When the phosphotungstic acid precipitate separates in abundance, we have frequently noticed that it forms a relatively stiff jelly which, on stirring, diminishes greatly in volume and after a time becomes partly crystalline, as shown by the microscope. The same phenomenon, though less marked, usually occurs on washing, the precipitate diminishing in volume and becoming more crystalline, but not, so far as we have observed, dissolving to a noticeable extent.

That the amount of nitrogen precipitated by phosphotungstic acid varies with the conditions, is evident from the facts brought

[1] *Ztschr. physiol. Chem.*, **31**, 165 (1900).

forward by Kutscher, who, however, worked under extreme conditions, his solutions being very concentrated and the amount of phosphotungstic acid very large, so that coprecipitation of the monamino acids might be expected.

To determine the best way of employing Hausmann's method, we have tried the following experiments with a very pure preparation of crystallized edestin from the hemp seed:

I. One gram of the air-dry crystals of edestin was boiled for about seven hours with 12 per cent. hydrochloric acid, the solution evaporated to a small volume in order to remove most of the excess of hydrochloric acid, diluted with water, distilled with magnesia and the ammonia determined. The residual solution was then filtered, concentrated to approximately 100 cc., 5 grams of sulphuric acid added and then, after cooling, a solution of phosphotungstic acid of unknown strength, until no further precipitation occurred at once on adding more of the reagent to the clear solution above the precipitate. After twenty-four hours, the precipitate was filtered out and washed with a dilute solution of phosphotungstic acid containing some sulphuric acid. Nitrogen was then determined in the precipitate together with the filter.

II. One gram of the same preparation of edestin was dissolved in dilute hydrochloric acid, somewhat more than an equal volume of concentrated acid added and the solution boiled with a reflux condenser for seven hours. After boiling a short time, the excess of hydrochloric acid passed out of the condenser so that the solution soon came to contain about 20 per cent. of the acid. The solution was then treated like I, and the nitrogen determined in the residue, filtered out after distilling off the ammonia. The filtered solution was then concentrated to 100 cc., 3 cc. of concentrated sulphuric acid added and, after cooling to about 20°, a solution containing 20 grams of phosphotungstic acid and 5 grams of sulphuric acid per 100 cc. was added, 1 or 2 cc. at a time, as long as an immediate precipitate formed in the solution cleared by subsidence. For this purpose, 15 cc. of the phosphotungstic acid solution were required. After standing half an hour, this precipitate was filtered out and 15 cc. more phosphotungstic acid were added. The solution remained clear for a few seconds and then slowly yielded a second voluminous precipitate which the microscope showed to consist of spherical aggregates of narrow crystalline plates together with amorphous matter entangled in a

very voluminous jelly. No noticeable difference existed in the appearance of this precipitate and that first thrown down. On stirring, the voluminous precipitate, which at first filled nearly the whole of the solution, settled rapidly to a slight crystalline deposit. After standing several hours, the clear solution was decanted and 35 cc. more of the phosphotungstic acid solution were added. After standing over night, a mere trace only of the precipitate separated from this last solution. The two precipitates first formed were washed with a solution containing 2.5 grams of phosphotungstic acid and 5 grams of sulphuric acid per 100 cc. and nitrogen determined in them.

III. One gram of the edestin crystals was treated in the same way as II, except that after adding the first 15 cc. of phosphotungstic acid the solution was allowed to stand twenty-four hours before filtering, no more phosphotungstic acid being added.

IV. One gram of the edestin was treated like III, except that 30 cc. of the phosphotungstic acid were used. The filtrate from the precipitate thus produced was made up to 500 cc. and nitrogen determined in 100 cc. of it.

V. One gram of the edestin was treated like III except that 60 cc. of the phosphotungstic acid were added.

The results of these determinations in per cent. of the edestin dried at 110° were the following:

	I.	II.	III.	IV.	V.
Phosphotungstic acid added..	? cc.	15 cc. + 15 cc.	15 cc.	30 cc.	60 cc.
Nitrogen as ammonia	1.93	1.86	1.86	1.86	1.86
Basic nitrogen	5.68	5.05 0.70	5.39	5.98	6.06
Non-basic nitrogen	10.23	...	...	10.01	...
Nitrogen in magnesium oxide precipitate	...	0.11	0.11	0.13	0.11
	17.94			17.98	

These figures show that the amount of ammonia formed is very uniform, even though the strength of the acid varies considerably,—from 12 to 20 per cent. The amount of basic nitrogen found in III shows that 15 cc. of the phosphotungstic acid is not enough, a result confirmed by the figures under II, where the addition of 15 cc. more phosphotungstic acid to the filtrate from the precipitate produced by the first 15 cc. yielded a second precipitate containing 0.7 per cent. more nitrogen. As 60 cc. of

phosphotungstic acid in V gave the same result as 30 cc. in IV, it would appear that the latter quantity was sufficient for complete precipitation and that a considerable excess of the precipitant has no solvent action, as might have been expected from Kutscher's statements.

The sum of the different forms of nitrogen falls considerably below the total nitrogen contained in edestin, namely, 18.64 per cent. This deficiency probably falls, for the most part, on the non-basic nitrogen, as it is difficult to determine this with accuracy, because only one-fifth of the solution can be employed and consequently all errors arising from incomplete oxidation during digestion with sulphuric acid are multiplied by five. Owing to the large amount of phosphotungstic acid present, larger quantities of the solution cannot well be used, and even with one-fifth, the solution bumps so badly, on boiling with sulphuric acid, that it is very difficult to effect complete conversion of the nitrogen into ammonia. It is better, therefore, to omit this determination and find the amount of non-basic nitrogen by difference.

It is evident from the results of these comparative determinations that a considerable latitude in the conditions may occur without noticeably affecting the result, so that uniform results may be obtained by using Hausmann's method under suitably defined conditions.

The method which we have adopted is the following: About 1 gram of the protein is boiled with 20 per cent. hydrochloric acid until the solution no longer gives the biuret reaction, usually from seven to ten hours. It is then evaporated on the water-bath to 2-3 cc. and the bulk of the free hydrochloric acid thus removed. The residual solution is transferred to a flask with about 350 cc. of water, and a cream of magnesia which has been freed from every trace of ammonia by long boiling is added until in slight, but distinct excess. After distilling and determining the ammonia, the solution in the flask is filtered through nitrogen-free paper and the residue, thus collected, washed thoroughly with water, and nitrogen determined in it, together with the paper, by Kjeldahl's method. The filtered solution is next concentrated to 100 cc., cooled to 20°, 5 grams of sulphuric acid added, and then 30 cc. of a solution containing 20 grams of phosphotungstic acid and 5 grams of sulphuric acid per 100 cc. After twenty-four hours, the precipitate is filtered out and washed with a solution containing

2.5 grams of phosphotungstic acid and 5 grams of sulphuric acid per 100 cc. The washing is effected by rinsing the precipitate from the filter into a beaker and returning to the paper three successive times, each portion of the wash-solution being allowed to run out completely before the next is applied. About 200 cc. of washings are thus obtained.[1]

The nitrogen contained in the precipitate is then determined by transferring it to a flask of Jena glass holding 600 cc. and digesting with 35 cc. of sulphuric acid for seven or eight hours. During the digestion, potassium permanganate crystals are added three or four times. In the few cases, where the phosphotungstic acid precipitate is small, less sulphuric acid is used, enough being taken in each case to prevent too violent bumping. The remaining nitrogen, belonging chiefly to monamino acids, is found by subtracting the sum of the nitrogen found in the preceding operations from the total nitrogen contained in the protein under examination.

THE BINDING OF NITROGEN IN VARIOUS PURE PROTEINS.

In studying the amount of furfurol yielded by various protein substances when distilled with 12 per cent. hydrochloric acid, we obtained solutions containing their decomposition products in which we determined the proportion of the different groups of nitrogen compounds in the same way as described in Experiment I with edestin. In the following pages these results are marked *a*.

We later studied Hausmann's method more carefully and, after trying the experiments already described, adopted the method just given. The results thus obtained are marked *b*.

In nearly all of these proteins we determined the amount of non-basic nitrogen directly, but as in many cases the sum of the nitrogen in the several groups of compounds fell below the total contained in the preparation, we give in the following pages the amount found by difference, which we consider to be more nearly correct than that found by direct determination.

Edestin.

The name edestin was first applied to the globulin obtained by

[1] Hausmann directed that the washing be continued until the fluid ran out colorless, but in all the solutions which we precipitated with phosphotungstic acid there was either no coloring-matter at all or so little that the solution was, at the most, only a pale straw color. This was probably due to precipitation of humus by magnesia.

the writer from several different seeds[1] and in this paper he says that "this substance (the globulin of the cotton seed) agrees in composition with the vitellin which exists in the seeds of wheat, maize, hemp, castor bean, squash and flax. As the properties of the preparations obtained from all these sources are substantially alike, there can be little doubt that one and the same proteid exists in them all. For this body we adopt the name *edestin*, from the Greek edestos, signifying edible, in view of its occurrence in so many important food-stuffs."

In an earlier paper on "Crystallized Vegetable Proteids"[2] the writer compared the crystallized globulins from the hemp seed, castor bean, flax seed and squash seed and showed that in crystalline form, composition and reactions the various preparations were practically alike. In conclusion he stated: "It is impossible to assert that these four globulins are the same, but, since differences exist between different preparations of globulin from the same seed as great as those found among the globulins of these different seeds, the writer is disposed to consider these four globulins as identical."

As it has since been found that most of the differences above referred to are caused by different minute proportions of combined acid,[3] whereby different salts of the protein were formed, it seemed even more probable that these four globulins were one and the same substance.

In another paper by Osborne and Campbell[4] the supposition was advanced, based on analyses by Chittenden, that the globulin of the cocoanut, which is obtained in crystals, is probably edestin.

In a paper on the proteids of the sunflower seed, Osborne and Campbell[5] state that "it is therefore our opinion that the sunflower seed contains, as its principal proteid, the globulin edestin, but that, as obtained by extraction from the seed, this is mixed with helianthotannic acid, from which we have not succeeded in separating it completely."

We have now determined the different forms of nitrogen in such of these preparations as are at present available, with the following results:

[1] Report Conn. Agr. Expt. Station for 1893 and this Journal, **16**, 778 (1894).

[2] *Am. Chem. J.*, **14**, 662 (1892).

[3] Report of Conn. Agr. Expt. Sta. for 1896, p. 369, and this Journal, **19**, 482 (1897); also this Journal, **21**, 486 (1899) and Report Conn. Agr. Exp. Sta. for 1900, p. 399.

[4] Report Conn. Agr. Expt. Sta. for 1895, p. 288, and this Journal, **18**, 609.

[5] Report Conn. Agr. Expt. Sta. for 1896, p. 374, and this Journal, **19**, 487.

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN PROTEINS HERETOFORE SUPPOSED TO BE EDESTIN.

Source.			Nitrogen as NH_3.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Hemp seed (*Cannabis sativa*)		*a*	1.93	5.68	11.04	...	18.64
		b	1.86	5.98	10.68	0.13	....
		c	1.86	6.06	10.62	0.11	....
Cotton seed (*Gossypium herbacium*)		*a*	1.92	5.71	11.01	...	18.64
Cocoanut (*Cocos nucifera*)		*a*	1.34	6.02	11.05	0.07	18.48
		b	1.38	6.11	10.79	0.20	....
Castor bean (*Ricinus communis*)		*a*	1.96	5.64	11.03	0.12	18.75
Flax seed (*Linum usitatissimum*)	I[1]	*a*	2.06	4.77	11.60	0.05	18.48
	II	*b*	1.89	4.68	11.64	0.27	....
	III	*b*	1.97	4.63	11.64	0.17	....
	IV	*b*	2.04	5.15	10.88	0.33	....
	V	*b*	2.04	4.56	11.66	0.22	....
	VI	*b*	2.00	4.86	11.37	0.25	....
Squash seed (*Cucurbita maxima*)	I	*b*	1.36	5.93	11.04	0.18	18.51
	II	*b*	1.21	6.04	11.01	0.25	....
	III	*b*	1.26	5.94	11.08	0.23	....
Sunflower seed (*Helianthus annus*)		*a*	2.55	4.33	11.46	0.24	18.58
		b	2.58	4.21	11.55	0.24	....
Wheat kernel (*Triticum vulgare*)	I	*b*	1.49	6.66	10.04	0.20	18.39
	II	*b*	1.35	7.01	9.60	0.35	....

These figures show that the globulins from hemp seed, cotton seed and castor beans contain practically the same proportion of nitrogen in each of the different forms; that the globulins of the cocoanut and squash seed are very nearly alike but contain so much less nitrogen as ammonia than the globulins of the three seeds first named that there can be no doubt that they are distinctly different proteins; that the globulin of the flax seed and that from the sunflower seed contain very much less basic nitrogen than the others, but that the former is distinguished from the latter in yielding 0.5 per cent. less nitrogen as ammonia. The globulin from wheat is characterized by containing by far the largest proportion of basic nitrogen of any of these globulins and far more than any of the other proteins described in the following pages.

[1] All sets of figures used in this paper which are marked with Roman numerals were obtained from different preparations; those showing more than one determination on the same preparation are included in brackets.

We have not been able to determine the proportion of the different forms of nitrogen in the globulins of seeds of rye, barley and maize, which are very similar in properties and composition to that of wheat, and can therefore assert nothing as to the relations of these apparently identical proteins.

The facts here presented raise the question as to which of the above proteins should now be called edestin. Although the writer first applied the name *edestin* to the globulin of the cotton seed together with that "which exists in the seeds of wheat, maize, hemp, castor bean, squash and flax" he always considered that the crystallized globulin from the four seeds last named best represented this substance. We now find that only the globulins of hemp seed, castor bean and cotton seed are alike in respect to the proportion of their several nitrogenous decomposition products. In another paper we show that the globulin from cotton seed gives a strong Molisch reaction while those from the hemp seed and castor bean give none at all. We have then only two of the above globulins, with the possible exception of those of wheat and maize, which can still be considered alike, namely those from the hemp seed and castor bean. It seems best, therefore, to retain the name edestin for the globulin of these latter seeds, especially as it is now very generally applied to that from hemp seed. Whether the globulins from these two seeds are in fact alike is rendered doubtful by the other results of this investigation, for only those proteins appear to be identical that originate from seeds which are closely related botanically, *e. g.*, legumin from the pea, horse bean, lentil and vetch, vicilin from the pea, horse bean and lentil, gliadin from wheat and rye, and phaseolin from the kidney bean and adzuki bean.

Legumin.

In the seeds of the pea, lentil, horse bean and vetch, Osborne and Campbell[1] studied the protein substance legumin, which, so far as a rigid comparison of the properties and composition of preparations from each of these seeds could show, appeared to be one and the same protein. A determination of the different forms of nitrogen in legumin from these four seeds has given the following results:

[1] Report of the Conn. Agr. Expt. Sta. for 1897, pp. 324, 337, 393, 361, and this Journal, **20**, 348, 362, 393, 406 and 419.

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN LEGUMIN.

Source.		Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Pea (*Pisum sativum*)	*b*	1.66	5.24	10.74	0.27	17.91
	b	1.72	5.43	10.56	0.20	....
Lentil (*Ervum lens*)	*b*	1.69	5.16	11.03	0.11	17.99
Horse bean (*Vicia faba*)	*b*	1.62	4.92	11.34	0.11	17.99
Vetch (*Vicia sativa*)	*b*	1.75	5.17	10.90	0.18	18.00

In view of these results, and the fact that legumin from each of these seeds has the same composition and reactions, there can be little doubt as to the identity of the several preparations.

Phaseolin.

The writer has found that the chief protein in the seeds of the kidney bean is a globulin, to which he gave the name phaseolin.[1] Later Osborne and Campbell found[2] in the Japanese adzuki bean a globulin which was so like phaseolin, both in properties and composition, that it was stated to be that protein. They also found in the soy bean[3] a protein similar in properties and composition to phaseolin, in regard to which they say, "The soy bean meal contains a more soluble globulin which resembles phaseolin in composition and, so far as we could ascertain, also in its reactions. The amount of this protein is small and the evidence that it is in reality phaseolin is not wholly satisfactory."

The following table contains the determinations of the different forms of nitrogen yielded by a number of fractional precipitates of phaseolin from the kidney and adzuki beans and also by preparation *10* from the soy bean.

[1] Report Conn. Agr. Expt. Sta. for 1893, p. 186, and this Journal, **16**, 633, 703 and 757.
[2] Report Conn. Agr. Expt. Sta. for 1896, p. 387, and this Journal, **19**, 509.
[3] Report Conn. Agr. Expt. Sta. for 1897, p. 374, and this Journal, **20**, 419.

PERCENTAGE OF NITROGEN IN THE DIFFERENT FORMS IN PHASEOLIN.

Source.			Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Kidney bean (*Phaseolus vulgaris*)	I	*b*	1.63	3.65	10.54	0.38	16.20
	II	*a*	1.74	3.48	....	...	....
		b	1.77	3.85	10.29	0.29	....
	III	*b*	1.63	3.51	10.74	0.32	....
Adzuki bean (*Phaseolus radiatus*)	I	*b*	1.72	...	....	...	....
		b	1.69	4.32	9.99	0.20	...
	II	*b*	1.78	...	....	0.38	....
		b	1.69	4.10	10.15	0.26	....
	III	*b*	1.75	4.19	9.82	0.44	....
		b	1.77	4.24	10.03	0.16	....
	IV	*b*	1.70	...	....	0.30	....
		b	1.69	4.17	10.16	0.18	....
	V	*b*	1.76	4.11	10.05	0.28	....
		b	1.86	4.13	....	...	....
Soy bean (*Glycine hispida*).....	10	*b*	1.78	3.83	10.22	0.37	....
		b	1.97	4.02	9.99	0.22	....

Although the amount of basic nitrogen found in the phaseolin from the adzuki bean is distinctly and uniformly more than in that from the kidney bean, we are not inclined to consider the difference, which hardly exceeds the error of analysis, to be sufficient to warrant the conclusion that the globulins from these two seeds are different substances, especially as no other difference of any sort has been found between them.

Legumelin.

In most of the leguminous seeds which have been examined in this laboratory, a protein has been found which is soluble in exceedingly dilute saline solutions, is coagulated at about 60° and is obtained by dialyzing in alcohol the solutions previously freed from globulin by dialysis in water. It is thus separated in a coagulated form and can be freed from proteoses and other soluble, contaminating substances by washing with water. The properties, composition and occurrence of legumelin are given in a paper by Osborne and Campbell.[1]

The proportion of the different forms of nitrogen in legumelin is given in the following table, the designations of the prepara-

[1] Report Conn. Agr. Expt. Sta. for 1897, p. 365, and this Journal, **22**, 410.

tions being those of products whose analyses were given under the same numbers in the papers already referred to.

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN LEGUMELIN.

Source.			Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Pea (*Pisum sativum*)	41	*b*	1.04	...	....	...	....
		b	1.02	3.75	10.87	0.48	16.12
	42	*b*	1.04	4.05	10.84	0.28	16.21
Lentil (*Ervum lens*)	75	*b*	1.04	3.69	10.88	0.45	16.06
	50	*b*	1.10	3.38	11.18	0.30	15.96
	66	*b*	1.11	3.70	10.93	0 51	16.25
Horse bean (*Vicia faba*)	96	*b*	0.96	3.42	11.10	0.44	15.92
Adzuki bean (*Phaseolus radiatus*)	6	*b*	0.96	3.94	10.89	0.31	16.10
		b	1.09	3.74	10.97	0.30	....

All these preparations are characterized by yielding a decidedly smaller proportion of nitrogen as ammonia than any of the other proteins examined, with the exception of leucosin, which will next be discussed. The results obtained agree fairly with one another and make it highly probable that legumelin from the several seeds is one and the same protein substance.

Leucosin.

In the seeds of wheat, rye and barley there is found a small quantity of a protein having the properties of an albumin, being soluble in water and coagulable by heat at 52°.[1] In the wheat kernel the leucosin is contained chiefly in the embryo, of which it forms about 10 per cent.,[2] while in the whole wheat, including the embryo, only about 0.3 or 0.4 per cent. is present.

The proportion of the different forms of nitrogen in leucosin were found to be as follows:

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN LEUCOSIN.

Source.		Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Wheat (*Triticum vulgare*)	I	1.18	3.58	11.76	0.41	16.93
	II	1.15	3.45	11.75	0.58	....
	III	1.16	3.48	11.99	0.30	....

[1] Osborne and Voorhees: *Am. Chem. J.*, **15**, 392 (1893); Osborne: Report Conn. Agr. Expt. Sta. for 1894, p. 147; this Journal, **17**, 429 (1895); Osborne: Report Conn. Agr. Expt. Sta. for 1894, p. 165; this Journal, **17**, 539 (1895).

[2] Osborne and Campbell: Report Conn. Agr. Expt. Sta. for 1899, p. 305.

These results are similar to those obtained for legumelin, with which protein leucosin agrees closely in composition, reactions and temperature of coagulation. Leucosin yields the smallest proportion of basic nitrogen of any of the proteins examined except glutenin and the alcohol-soluble proteins.

Vicilin.

In the seeds of the pea, lentil and horse bean there is a considerable quantity of a globulin associated with legumin, which is characterized by containing an extremely small proportion of sulphur. The properties of vicilin are given in a paper by Osborne and Campbell[1] and the proportion of nitrogen in different groups in the following table:

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN VICILIN.

Source.		Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Horse bean (*Vicia faba*)	*b*	1.93	4.53	10.35	0.23	17.04
Lentil (*Ervum lens*)	*b*	1.75	4.59	10.77	0.13	17.24
Pea (*Pisum sativum*)	*b*	1.67	5.12	10.00	0.26	17.05

These results, while not in quite as close agreement as most of our others, are, in our opinion, near enough to one another to warrant the conclusion that the preparations from the different seeds are identical. The higher result for basic nitrogen in vicilin from the pea is, perhaps, due to the fact that 1.5 grams, instead of 1 gram, was used by mistake, and of the horse bean and lentil vicilin only 0.75 gram of each was available for the determination. Unfortunately, no more of these preparations is left, so that the determinations cannot now be repeated.

Conglutin.

In the blue and yellow lupine, Osborne and Campbell[2] found globulin, in large proportion, which could be separated, by fractional precipitation, into two parts of different composition. The differences between the extreme fractions, in the case of the blue lupine, were only slight, suggesting the admixture or combination of some other substance. The differences between the extreme

[1] Report Conn Agr. Exp. Sta. for 1897, p. 365, and this Journal, **20**, 410.

[2] Report Conn. Agr. Expt. Sta. for 1896, p. 342, and this Journal, **19**, 454.

fractions from the yellow lupine were, however, so considerable that it was a question whether or not two different proteins were present in this seed. The least soluble fractions of the globulin from the yellow lupine agreed in composition and reactions closely with the corresponding fractions from the blue lupine and were designated *conglutin*. The most soluble fractions differed in reactions and composition, especially in sulphur content, those from the yellow lupine containing more than three times as much sulphur as those from the blue lupine. The results of that investigation indicated that some organic substance, rich in sulphur, was present in these seeds, much more in the yellow than in the blue lupine, which combined with the conglutin and could be separated only with difficulty. It was, however, found[1] that on treatment with hot alkaline solutions the same proportion of sulphur was split off as sulphide from the preparations rich in sulphur as from those poor in this element. As it seemed improbable that a contaminating sulphur-containing substance should contain the same proportion of sulphur convertible into sulphide as did the protein, it became a question whether or not there were two different proteins present. Hoping that we might get some evidence bearing on this point, we have determined the proportion of nitrogen in the different groups in several preparations from these seeds, with the following results, the designations of the preparations being the numbers of the preparations described in the papers referred to.

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN CONGLUTIN.

Source.		Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Blue lupine (*Lupinus angustifolius*)	37 *a*	2.12	5.52	10.21	0.09	17.94
Yellow lupine (*Lupinus luteus*)	2 *a*	2.18	5.25	10.36	0.05	17.84
	23 *b*	2.14	4.90	10.65	0.24	17.93
	23 *b*	2.00	5.06	10.65	0.22	....
	26 *b*	2.22	5.24	10.10	0.24	17.80
	28 + 29 *b*	2.04	5.20	10.27	0.27	17.78
	30 + 31 + 32 *b*	2.68	5.20	10.30	0.03	18.21
	30 + 31 + 32 *b*	2.61	5.06	10.29	0.25	....

[1] Report Conn. Agr. Expt. Sta. for 1900, p. 443, and this Journal, **24**, 140.

Between the conglutin from the blue lupine and the less soluble no difference in the proportion of nitrogen belonging to the different groups. The more soluble globulin of the yellow lupine, different groups. The more soluble globulin of the yellow lupine, which contains a much larger proportion of sulphur, yields a distinctly greater amount of ammonia than do the other preparations, as shown by the figures given last in the table. Whether or not this excess of ammonia, as well as that of the sulphur, is caused by some non-protein substance combined with the conglutin of these fractions is not shown by these determinations.

Corylin.

In the hazel nut, *Corylus*, and English walnut, *Juglans regia*, is a large quantity of globulin to which the writer gave the name corylin.[1] We have recently obtained from the American black walnut a similar globulin which closely resembles that contained in the two nuts first mentioned. The proportion of nitrogen in the different groups in these globulins was found to be as follows:

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN CORYLIN.

Source.			Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Hazel nut (*Corylus*)	I	*a*	2.17	5.56	....	...	19.02
	II	*b*	2.22	5.94	10.70	0.16	....
Black walnut (*Juglans nigra*)	I	*a*	1.85	5.21	11.68	0.10	18.90
	II	*b*	1.71	5.61	11.33	0.19	....

The distinctly smaller proportion of ammonia nitrogen obtained from the black walnut globulin indicates a difference between these two proteins. The fact that these seeds are not closely related botanically, likewise makes this more probable and renders necessary a strict comparison of these two proteins with that of the English walnut, which will be made as soon as a supply of the globulin from the latter nut is obtained.

Other Plant Globulins.

The following table gives the results of our determinations of the proportion of the different forms in which the nitrogen was found in proteins which we have thus far obtained from the seeds of but one species of plants.

[1] Report Conn. Agr. Exp. Sta. for 1895, p. 288, and this Journal, **18**, 609 (1896).

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN VARIOUS PLANT GLOBULINS.

Protein. Source.		Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Excelsin—Brazil nut (*Bertholletia excelsa*)	*a*	1.45	...	....	...	....
	b	1.50	5.76	10.97	0.17	18.30
Amandin—Almond (*Prunus Amygdalus* var *dulcis*)	*a*	3.03	4.09	....	...	19.00
	b	3.06	4.21	11.55	0.18	....
Vignin—Cowpea (*Vigna catjang*)	*a*	1.91	4.28	10.81	0.25	17.25
	b	1.91	...	....	...	....
Glycinin—Soy bean (*Glycine hispida*)	*a*	2.11	4.00	11.26	0.08	17.45
	b	2.12	3.90	11.27	0.16	....
Avenalin—Oat (*Avena sativa*)	*b*	2.37	3.94	11.37	0.22	17.90

Amandin yields a larger proportion of nitrogen as ammonia than any of the other globulins and about the same proportion of basic nitrogen as the average of the majority of the other proteins examined. In this respect it differs from the other globulins rich in nitrogen which yield a much larger amount of basic nitrogen. Amandin must, therefore, have a decidedly different structure from these. The figures given in this table for the other proteins require no special comment.

NITROGEN IN THE DIFFERENT GROUPS IN ALCOHOL-SOLUBLE PROTEINS.

Kossel and Kutscher[1] have recently decomposed the alcohol-soluble proteins of wheat and maize kernels and have determined the proportion of ammonia, histidine and arginine which results. They found that lysin was entirely absent, although this diamino acid had been found in all the proteins previously examined, and also that the proportion of arginin was much less than that found in other proteins.

The results of our determinations by Hausmann's method of the different forms of nitrogen in several alcohol-soluble proteins are in harmony with the results obtained by Kossel and Kutscher, as the following table shows:

[1] *Ztschr. physiol. Chem.*, **31**, 165.

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN ALCOHOL-SOLUBLE PROTEINS.

Protein.	Source.			Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Gliadin	Wheat (*Triticum vulgare*)		*a*	4.20	1.18	....	...	17.66
			b	4.34	1.00	12.25	0.07	....
			b	4.36	...	....	0.20	....
Gliadin	Rye (*Secale cereale*)		*a*	3.94	0.95	....	...	17.72
			b	4.15	0.87	12.59	0.11	....
			b	4.22	0.92	12.39	0.20	....
Hordein	Barley (*Hordeum vulgare*)		*a*	3.96	0.66	....	...	17.21
			b	4.06	0.88	12.04	c.23	....
Zein	Maize (*Zea mays*)		*a*	2.95	0.67	12.46	0.05	16.13
			b	3.00	0.40	12.49	0.24	....
			b	2.98	0.48	12.45	0.22	....
			b	3.01	...	....	...	....
			b	2.93	0.40	12.68	0.12	....
			b	2.98	...	....	...	....
Alcohol-soluble protein	Oat (*Avena sativa*).....	I	*b*	3.55	1.04	10.85	0.26	15.70
			b	3.67	1.07	10.68	0.28	....
		II	*b*	3.37	1.46	10.59	0.23	15.65
			b	3.50	1.38	10.56	0.21	....
Bynin	Malt (*Hordeum vulgare*)...	I		3.79	0.80	11.11	0.56	16.26
		II		3.86	0.70	11.23	0.47	....

Kossel and Kutscher[1] made determinations of the diamino acids in preparations believed to represent the three alcohol-soluble proteins, mucedin, gliadin, and gluten fibrin, which Ritthausen[2] described as constituents of wheat gluten, and state that their preparations were made according to Ritthausen's directions. Unfortunately, the writer has never been able to understand Ritthausen's explanation of the process by which these three proteins were prepared, although he has devoted much time and attention to its description. We have been unable, therefore, to prepare products which could be considered to fairly represent these three proteins, but the very extensive study of a large number of fractions obtained from alcoholic solutions of every possible strength failed to give Osborne and Voorhees[3] any evidence whatever of the existence in the wheat kernel of more than one protein which

[1] *Ztschr. physiol. Chem.*, **31**, 165.

[2] "Die Eiweisskörper, etc.," Bonn, 1872.

[3] *Am. Chem. J.*, **15**, 392.

is soluble in alcohol. Kjedahl[1] came to the same conclusion, as he says there can be only one alcohol-soluble protein in wheat since both composition and $[\alpha]_D$ are constant.

The only noticeable difference which Kossel and Kutscher found between their preparations of so-called gluten fibrin, gliadin and mucedin lay in the proportion of histidine. The amount of this substance found in their gliadin was a little less than in gluten fibrin and distinctly less in their mucedin than in the other two preparations. As only one determination was made in each of these products, the figures given require confirmation before the differences, which, as Kossel and Kutscher say, "are at the most only very slight," can be accepted as evidence of the presence of three alcohol-soluble proteins in the wheat kernel.

The nitrogen belonging to the histidine and arginine, which Kossel and Kutscher found, formed 1.39 per cent. of the gluten fibrin, 1.13 per cent. of the mucedin and 1.21 per cent. of the gliadin, which is equal to a mean of 1.24 per cent. in the three preparations. The amount of nitrogen which we precipitated by phosphotungstic acid from the solution of the decomposition products of gliadin falls a little below this figure, as would be expected from the known solubility of arginine phosphotungstate, of which this precipitate chiefly consisted. Gulewitsch[2] found when arginine is precipitated from 100 cc. of solution by phosphotungstic acid that 7 milligrams remain dissolved. This amount of arginine contains 2.2 milligrams of nitrogen which, if added to the average quantity found by us to be precipitated by phosphotungstic acid, gives 1.2 per cent. of basic nitrogen against 1.24 per cent. found by Kossel and Kutscher.

The amount of nitrogen in the arginine and histidine which Kossel and Kutscher found in zein was equal to 0.8 per cent. of the protein. If the amount of nitrogen corresponding to the solubility of arginine phosphotungstate is added to the average of our determinations of the basic nitrogen in zein, we have 0.71 per cent., which agrees very closely with Kossel and Kutscher's result.

The amount of nitrogen as ammonia which Kossel and Kutscher obtained from these alcohol-soluble proteins was decidedly less than that found by us, doubtless because a part of the ammonia was precipitated from their solutions with the humus substance.

[1] *Agriculturchem. Centrbl.*, **25**, 197 (1892).
[2] *Ztschr. physiol. Chem.*, **27**, 196.

These investigators also determined the proportion of diamino acids in the protein of wheat gluten that is insoluble in alcohol which they, following Ritthausen, call gluten casein, but which we prefer to call glutenin, since no near relation between it and the milk caseins exists, as was formerly supposed. The results of our determinations of the different groups of nitrogen in this protein were the following:

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN GLUTENIN.

Source.		Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Wheat gluten..	*b*	3.26	1.90	12.18	0.15	17.49
	b	3.35	2.20	11.71	0.23	....

Kossel and Kutscher found in glutenin 2.14 per cent. of nitrogen belonging to the diamino acids, histidine, arginine and lysine, with which figure the average of ours of 2.05, or, allowing for the solubility of arginine phosphotungstate, 2.27 is in good agreement. The amount of ammonia which they found was, as for the other proteins examined, less than that which we found, namely, 2.02 per cent. as against 3.31 per cent. found by us.

Kossel and Kutscher think that it is probable that, under the condition of their experiments, some of the ammonia unites with the humus, as suggested by Udransky,[1] and is estimated with the nitrogen of that substance.

Hart[2] has since found that on adding sodium chloride or sodium sulphate to the sulphuric acid with which he decomposed the proteins, that the amount of ammonia nitrogen was increased and that of the humus correspondingly diminished, which shows that a part of the ammonia is removed when the humus is separated from the solution before distilling with magnesia. By decomposing zein under these conditions, Hart found 2.65 per cent. of nitrogen as ammonia and none in the humus precipitate. That he found less than we is probably because he used barium carbonate instead of magnesia to expel the ammonia, though he does not say definitely that he did so. He has shown that barium carbonate yields less ammonia than

[1] *Ztschr. physiol. Chem.*, **12**, 42.

[2] *Ibid.*, **33**, 347.

magnesia, as the latter, according to his view, acts on some of the nitrogenous substances other than ammonium salts.

In conclusion we give some results, obtained with very pure preparations of casein, crystallized ovalbumin, conalbumin and nucleovitellin from the yolk of hen's eggs.

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN SOME ANIMAL PROTEINS.

Protein.		Nitrogen as ammonia	Basic nitrogen.	Non-basic nitrogen	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Casein	*a*	1.65	3.46	....	...	15.62
	b	1.58	3.53	10.30	0.21	....
Ovalbumin	*a*	1.35	3.22	....	...	15.51
	b	1.32	3.38	10.52	0.29	....
Conalbumin	*b*	1.23	4.10	10.57	0.21	16.11
	b	1.18	4.21	10.41	0.31	....
Nucleovitellin	*b*	1.21	4.60	10.28	0. 9	16.28
	b	1.28	4.69	10.07	0.24	....

The conalbumin was obtained by removing from an ammonium sulphate solution of egg white all of the crystallizable ovalbumin and then separating the protein substance which was deposited in spheroids by further evaporation. The aqueous solution of the latter substance was dialyzed in distilled water until all the ammonium sulphate was removed and the conalbumin coagulated by heating to 60°. The composition and properties of conalbumin are given in a recent paper by Osborne and Campbell,[1] whose observations respecting conalbumin have since been confirmed by Langstein.[2]

As conalbumin closely resembles ovalbumin in properties and composition, it seemed quite possible that it consisted simply of a compound of the latter with some other substance and that the protein part of the molecules of the two albumins might be the same. The results of our determinations show such wide differences in the proportion of basic nitrogen that there can be little doubt that these albumins are two distinctly different substances. Schulze[3] has come to the same conclusion as a result of his study of the "gold numbers" of these two albumins.

[1] Report Conn. Agr. Expt. Sta. for 1899, p. 348 : also this Journal, **22**, 422.

[2] Hofmeister's "Beiträge," **3**, p. 83 (1901).

[3] *Ibid.*, **3**, p. 157 (1902).

The amount of nitrogen as ammonia which we obtained from crystallized ovalbumin, namely, 1.35 per cent., is in very close agreement with the 1.32 per cent. found by Hausmann. Likewise we both found nearly the same amount of basic nitrogen, 3.22 and 3.31 per cent., whereas Kossel and Kutscher found considerably more, *i. e.*, 4.19. We have, unfortunately, been unable to find the conditions under which this last figure was obtained, it being quoted by Kutscher without reference to any publication. As the ovalbumin used does not appear to have been crystallized ovalbumin, it is possible that conalbumin may have contributed to the higher result.

Our determinations of nitrogen as ammonia yielded by milk casein agree with those calculated from Kutscher's figures, assuming that his casein contained 15.62 per cent. of nitrogen. Kutscher thus found 1.62, 1.62, 1.58 and 1.59 per cent. of nitrogen in this form. Hart[1] found 1.60 per cent. by distilling the decomposition products of casein with magnesia and 1.39 and 1.18 per cent. by distilling with barium carbonate the decomposition products obtained by boiling with sulphuric acid without adding sodium chloride and 1.52 per cent. after adding this salt.

Hart found that more lysine was obtained from the decomposition products of casein when the latter was boiled with sulphuric acid and sodium chloride than when boiled with sulphuric acid alone. He found, when using sulphuric acid and salt, an amount of histidine, arginine and lysine corresponding to 3.37 per cent. of basic nitrogen in the casein, which agrees closely with our result by Hausmann's method, namely, 3.49 per cent.

Gümbel[2] found, by Hausmann's method, nitrogen as ammonia corresponding to 1.62 per cent. of milk-casein and to 4.23 per cent. of basic nitrogen, the former being in close agreement with our results, but the latter considerably higher than those found by us or by Hart, who determined the bases directly.

If the results of the determinations by Hausmann's method, as employed by us, are compared with the results obtained by Kossel and Kutscher's method, in separating and determining the actual quantity of the diamino acids produced by decomposing comparatively large quantities of the protein substances, it will be seen that nearly the same amounts of basic nitrogen are obtained by both methods. In the following table we give the figures obtained by

[1] *Ztschr. physiol. Chem*, **33**, 347 (1901).

[2] Quoted by Hofmeister, "Ergebnisse der Physiol.," Vol. I, p. 777.

the two methods, the amount of nitrogen found by Hausmann's method being increased by the addition of a quantity of nitrogen corresponding to the solubility of arginine phosphotungstate.

PERCENTAGE OF BASIC NITROGEN IN SEVERAL PROTEINS.

Zein ..... { 0.80 by Kossel and Kutscher's method.
0.71 by Hausmann's modified method.

Gliadin ... { 1.24 by Kossel and Kutscher's method.
1.20 by Hausmann's modified method.

Glutenin . { 2.14 by Kossel and Kutscher's method.
2.27 by Hausmann's modified method.

Casein .... { 3.37 by Kossel and Kutscher's method.
3.71 by Hausmann's modified method.

These results indicate that Hausmann's method, as we have applied it, gives a fairly accurate measure of the true proportion of nitrogen belonging to the diamino acids. Before this can be demonstrated, however, quantitative determinations by Kossel and Kutscher's method must be made in other proteins, especially those yielding larger proportions of diamino acids.

An examination of the figures showing the different forms of binding of the nitrogen in the many proteins which we have investigated shows that these vary chiefly in the proportion of ammonia and basic nitrogen which they yield. In the following table we give the average of the figures found for each of these proteins, arranged in the order of amount of basic nitrogen.

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN VARIOUS PROTEIN BODIES.

Protein. Source.	Nitrogen as ammonia.	Basic nitrogen.	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Globulin—Wheat	1.42	6.83	9.82	0.28	18.39
" Cocoanut	1.36	6.06	10.92	0.14	18.48
" Squash seed	1.28	5.97	11.04	0.22	18.51
Edestin—Hemp seed	1.88	5.91	10.78	0.12	18.64
Excelsin—Brazil nut.....	1.48	5.76	10.97	0.17	18.30
Corylin—Hazel nut.....	2.20	5.75	10.70	0.16	19.00
Globulin—Cotton seed.....	1.92	5.71	11.01	...	18.64
" Castor bean.....	1.96	5.64	11.00	0.12	18.75
Corylin—Walnut	1.78	5.41	11.51	0.15	18.84
Conglutin—Lupine ..... *a*	2.12	5.20	10.38	0.18	17.90
b	2.65	5.13	10.30	0.14	18.21

Protein. Source.	Nitrogen as ammonia.	Basic nitrogen	Non-basic nitrogen.	Nitrogen in magnesium oxide precipitate.	Total nitrogen.
Legumin—Pea, lentil, horse bean, vetch	1.69	5.18	10.92	0.17	17.97
Globulin—Flax seed	2.00	4.77	11.47	0.22	18.48
Vicilin—Pea, lentil, horse bean	1.78	4.75	10.37	0.21	17.11
Nucleovitellin—Egg yolk	1.25	4.65	10.16	0.22	16.28
Vignin—Cow pea	1.91	4.28	10.81	0.25	17.25
Globulin—Sunflower	2.57	4.27	11.52	0.24	18.58
Conalbumin—Egg white	1.21	4.16	10.49	0.26	16.11
Amandin—Almond	3.05	4.15	11.55	0.17	19.00
Phaseolin—Kidney bean, adzuki bean	1.74	3.97	10.18	0.29	16.20
Glycinin—Soy bean	2.11	3.95	11.27	0.12	17.45
Legumelin—Pea, lentil, horse bean, adzuki bean	1.04	3.71	10.96	0.38	16.09
Leucosin—Wheat	1.16	3.50	11.83	0.43	16.93
Casein—Cow's milk	1.61	3.49	10.31	0.21	15.62
Ovalbumin—Egg white	1.34	3.30	10.58	0.29	15.51
Glutenin—Wheat gluten	3.30	2.05	11.95	0.19	17.49
Gliadin—Wheat, rye	4.20	0.98	12.41	0.14	17.66
Hordein—Barley	4.01	0.77	12.04	0.23	17.21
Zein—Maize	2.97	0.49	12.51	0.16	16.13

The most striking feature shown by this table is the wide range in the amounts of basic nitrogen obtained from the different proteins. While the difference between the highest total nitrogen and the lowest is 3.49 per cent. of the protein, or 18.3 per cent. of the highest nitrogen, that between the highest basic nitrogen and the lowest is 6.34 per cent., a difference of 92.7 per cent. of the highest figure. The proportion of ammonia yielded by these different proteins likewise differs greatly, the difference between the highest and lowest figures being 3.16 per cent. or 75.2 per cent. of the highest figure. The non-basic nitrogen, on the other hand, is much more constant even than the total nitrogen, the difference between the highest and lowest being only 2.69 per cent. of the protein or 21.5 per cent. of the highest figure.

Apart from the alcohol-soluble proteins, which all come together at the end of the table, no other relation depending on the proportion of basic nitrogen is apparent.

The crystalline globulins from the hemp seed, squash seed and flax seed, as we have already said, are so nearly alike in solubility, reactions, crystalline form and composition that a most rigid comparison has as yet failed to reveal any differences which indicated that they are not one and the same chemical individual: nevertheless the globulin from the flax seed differs from the other two in the amount of basic nitrogen which it yields by over 1 per cent. and the globulin from the squash seed differs in the amount of ammonia which it yields by about 0.6 per cent. The molecules of these globulins evidently have a different structure.

In the table, all of the proteins down to legumelin are globulins, with the exception of the nucleovitellin, which, however, as obtained originally from the egg yolk in combination with lecithin, has the properties characteristic of globulin, but after washing with alcohol it passes into the condition in which it was used for these experiments, in which it is no longer soluble in saline solutions. Legumelin and ovalbumin, the proteins next following in the table, are soluble in water and in this respect differ from those preceding them, but no difference in the proportion of basic nitrogen exists between legumelin and phaseolin, which is a pronounced globulin, and ovalbumin does not contain very much less.

Those proteins which are characterized by dissolving in strong alcohol present, on the other hand, a marked contrast to the others, in that they all yield far less basic nitrogen and more ammonia than the others, with the single exception of amandin which yields the same amount of ammonia as does zein but over eight times as much basic nitrogen.

The larger proportion of nitrogen which characterizes so many of the proteins of seeds, compared with the nitrogen in animal proteins, appears to be caused by a larger proportion of substances yielding ammonia and basic products. Some of the plant globulins contain nearly as much basic nitrogen as corresponds to the nitrogen content of the histidine, arginine and lysine which Kossel and Kutscher found in the histone from the thymus gland, namely, 6.43 per cent., while the globulin of wheat contains even more. If, as seems probable, the basic nitrogen of these vegetable proteins shall be shown by further investigation to belong wholly to the three diamino acids named, it would appear that the basic properties of the proteins are not caused simply by the diamino acid

components of their molecules, as Kossel and Kutscher suggest, for the histones are much stronger bases than any of these vegetable proteins.

This wide variation in the proportion of basic decomposition products of the various proteins, as Kossel and Kutscher point out, raises important questions regarding their food value.

At Kossel's suggestion, Szumoski,[1] after feeding geese and doves with maize for long periods, examined their various organs and tissues for zein, with negative results. That, however, zein is, in fact, assimilated is, in Szumoski's opinion, proved by the experiments which Grandeau, Leclerc and Ballacey[2] made with horses, and Rubner[3] made with men.

Feeding experiments with "gluten meal" present much stronger evidence on this point, since they show that the proteins of this meal are quite as well assimilated as those of cotton seed meal.

The "gluten meal" used in these experiments is a product of the manufacture of maize starch and contains a large proportion of the alcohol-soluble zein. The proportion of alcohol-soluble zein has not been accurately determined and doubtless varies with the different samples, but in a large number examined at different times by the writer not far from 25 per cent. of these meals were found to consist of alcohol-soluble zein.

The digestibility of the proteins of gluten meal has been found, as the average of several experiments, to be 88.2 per cent., while that of cotton seed meal is 88.4 per cent.,[4] from which it is evident that the proteins of gluten meal possess a high coefficient of digestibility, and as these consist largely of zein, it is almost certain that zein is assimilated without special difficulty.

In order to show the relative proportions of the several groups of nitrogenous decomposition products yielded by these meals when treated with boiling acids, we took a portion of each meal containing 0.1600 gram of nitrogen, and treated it in exactly the same way as described in this paper for the proteins. The results were as follows:

[1] *Ztschr. physiol. Chem.*, **36**, 198.
[2] *Ann. de la Science Agronomique*, **9**, Ann., T. I., 1892.
[3] *Ztschr. Biol.*, **15**, 150 (1879).
[4] Bulletin 77, Office of Experiment Stations, U. S. Dept. of Agr.

PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN THE PROTEINS OF THE MEAL, ASSUMING THESE TO CONTAIN 16 PER CENT. OF NITROGEN.

	Cotton seed meal.	Gluten meal.
Nitrogen as ammonia	1.52	2.38
Basic nitrogen	4.97	1.42
Non-basic nitrogen	8.67	11.63
Nitrogen in magnesium oxide precipitate	0.84	0.57
Total nitrogen	16.00	16.00

These figures show how great the difference is between the proportions of these several nitrogenous groups and, since no apparent difference in food value exists between these meals, it would seem as if, from the standpoint of nutrition, these very decided chemical differences were of but little importance.

Since zein contains 16 per cent. of nitrogen, the figures given for the gluten meal may be directly compared with those of zein. As the globulin of the cotton seed contains 18.6 per cent. of nitrogen, the amount of meal taken corresponded to only 0.8608 gram. If we calculate the figures given for cotton seed meal to this basis, they become comparable with those of the cotton seed globulin. In the following table the results of this comparison are shown.

	Protein in cotton seed meal.	Globulin of cotton seed meal.	Zein.	Protein in gluten meal.
Nitrogen as ammonia	1.77	1.92	2.97	2.38
Basic nitrogen	5.77	5.71	0.49	1.42
Non-basic nitrogen	10.12	11.01	12.51	11.63
Nitrogen in magnesium oxide precipitate	0.98	....	0.16	0.57
Total nitrogen	18.64	18.64	16.13	16.00

From these figures it appears that the total protein of the cotton seed meal yields practically the same proportion of decomposition products as the cotton-seed globulin, the differences shown being unquestionably due to the greater amount of humus arising from the carbohydrates, whereby a larger amount of nitrogen appears in the "MgO pp." and a smaller amount as ammonia. In the case of gluten meal, it is evident that some protein other than zein is also present, but its proportion is not indicated by the figures. Loewi[1] has just shown that a dog can be kept in nitrogenous

[1] *Archiv. f. Exper. Path. u. Pharm.*, **48**, 303 (1903).

equilibrium or even gain nitrogen when fed with food containing protein decomposition products which are wholly free from any substance giving the biuret reaction, that is, with food containing no protein whatever. The animal can therefore synthesize protein from a mixture of the crystallizable products produced by decomposition of protein. Since such a wide difference exists between the proportions in which the several groups of products are yielded by the different food proteins, this synthesis must consist in something more than a recombination of the several fractions of the molecule of the food protein; it must involve a more or less extensive alteration of these fractions and conversion of one into another before the requisite number of groups of proper nature are at hand from which the new molecule can be constructed.

If we consider the probable number of these groups and the many kinds of them which must take part in this synthesis, the selective and constructive power of the cells in which this process takes place appears to be very great. Hofmeister[1] states that if a mean molecular weight of 130-140 is assumed for the splitting products of the protein molecule, there must be at least 40 such groups in the protein molecule if its molecular weight is 5,000, or 120 groups if it is 15,000.

There are already about twelve different kinds of these groups known which are primary decomposition products of the protein molecule. The complexity of the process whereby the new protein molecule is constructed from the decomposition products of the food protein is thus easily apparent.

The fact that so many of the vegetable proteins, which serve extensively as food, have been shown, by our present investigation, to yield such different proportions of the various nitrogenous decomposition products, as compared with the animal proteins, makes it a matter of the greatest interest and importance to know something more of the processes involved in this synthesis.

[1] "Ergebnisse der Physiol.," Vol. I, p. 774.