LEGUMIN AND OTHER PROTEIDS OF THE PEA AND THE VETCH.[1]

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Received May 21, 1896.

LEGUMIN.

UNDER the name Legumin, many preparations, obtained from various seeds, have been described, but in such different and often conflicting terms as to leave us completely in doubt with regard to the nature of this substance.

This confusion appears to have arisen largely through the mistaken idea, which formerly was very generally held, that all the proteids extracted from seeds by water and precipitated by acids are one and the same substance.

The methods of analysis employed by the earlier chemists were too crude or uncertain to set forth the slight differences in composition of the various plant proteids, and the difficulty of making pure preparations tended, as the subject was further studied, to add to the confusion. Since the methods of analysis have been perfected and the more recently developed modes of studying proteids introduced, legumin has received little or no attention. In recent literature legumin is most commonly referred to as a substance extracted from seeds by caustic alkalies, and more or less altered by the action of the solvent, but nothing has been done, to our knowledge, to show the nature of the original proteid.

The object of our investigation has been to examine the seeds in which legumin is said to exist and to determine as definitely as possible the composition and character of this substance.

In 1806 Einhof[2] recognized a proteid in beans and lentils which he considered to be different from the bodies of this class previously known.

Braconnot[3] named this substance legumin.

Noad[4] prepared and analyzed legumin from peas and beans.

Norton[5] prepared legumin from peas, sweet almonds, and oats, and gave analysis of his preparations.

[1] From the Report of the Connecticut Agricultural Experiment Station for 1895. Communicated by the authors.

[2] Gehlens: *J. d. Chem.*, 6, 543.

[3] *Ann. de Chim. et de Phys.*, 34, [2] 68, *1827*.

[4] *Chem. Gaz.*, *1847*, 357.

[5] *Am. J. Sci.*, [2], 5, 22, *1847*.

Loewenberg[1] considered that legumin, as previously prepared, contained albumin and devised a method for the separation of these two proteids and gave analyses of the substances so prepared from almonds and peas.

Liebig[2] obtained plant casein (legumin) from beans, lentils, and peas, and gave an account of the properties of this proteid and two analyses. He concluded that the substance was identical in properties and composition with milk casein.

Dumas and Cahours[3] prepared legumin from peas, lentils, beans, almonds, plums, filberts, and white mustard. They considered all these seeds to contain the same proteid substance; that obtained from the three first named seeds being less pure than that from the others and therefore containing somewhat less nitrogen.

They gave analyses of preparations from all these seeds and an extended account of the properties of legumin, based on a study of the preparation obtained from the almond.

Contrary to Liebig, they concluded that this substance is not identical either with milk casein or plant casein. The latter designation they applied to the body which separates out on cooling a concentrated hot alcoholic extract of wheat gluten.

Rochleder[4] pointed out that the substance obtained from beans, lentils, and peas by Liebig was different from that of the almond described by Dumas and Cahours, and that for this reason these investigators did not reach the same conclusions. Rochleder prepared and analyzed legumin from two varieties of beans.

In 1868 Ritthausen undertook a study of legumin, the results of which are recorded in a series of papers whose publication extended over a period of fifteen years.[5]

He recognized that the seeds of almonds, plums, filberts, and white mustard, which had been previously stated to yield legumin, really contain a different proteid, which he called *conglutin*.

[1] *Ann. Phys.*, Pogg., **78**, 327.

[2] *Ann. d. Chem. u. Pharm.*, **39**, 138.

[3] *J. prakt. Chem.*, **28**. 398.

[4] *Ann. d. Chem. u. Pharm.*, **46**, 153.

[5] *J. prakt. Chem.*, **103**, 65, *1868*: Die Eiweisskörper, etc., Bonn., *1872*: Pflüger's Archiv., **15**, 269, *1877*: *Ibid*, **16**, 293, *1878*: *Ibid*, **18**, 236, *1781*: *J. prakt. Chem.*, [2], **24**, 221, *1881*; *Ibid*, [2], **26**, 504, *1882*.

Up to this time legumin was considered to be the proteid that is extracted from seeds with water and is precipitated by acids from the aqueous extract.

All proteids thus obtained had been regarded as identical by most investigators and were known either as legumin or plant casein. Although it had been suggested that different seeds yield different proteids, Ritthausen appears to have been the first to make this fact evident. Ritthausen prepared "legumin" from blue lupins, yellow, green, and gray field peas, yellow garden peas, lentils, vetches, horse beans (Vicia faba), white and yellow beans (Phaseolus) and colza cake. The proteid of Phaseolus, Ritthausen afterwards found to be distinct from legumin, and one of us has, in the main, confirmed his later result and has named the proteid phaseolin.[1] Ritthausen afterwards considered the proteid which he obtained from colza cake to be an impure preparation of a different substance. His early analyses[2] of preparations from the leguminous seeds were fairly accordant, but he afterwards found that the soda lime method which was used in determining nitrogen gave too low results. He thereupon determined nitrogen anew by Dumas' method, and published a revised statement of the mean composition of legumin.[3]

In another paper published shortly afterwards, Ritthausen withdrew the corrected figures for nitrogen, having found that they were too high, because the nitrogen of his later analyses was mixed with hydrogen. He therefore published a third set of figures for nitrogen and made a second revised statement of the mean composion of legumin.[4]

At this time Hoppe-Seyler[5] and Th. Weyl[6] stated that the proteids of plants are chiefly globulins and Weyl examined qualitatively a number of seeds, by extracting them with ten per cent. sodium chloride solution, and found proteids resembling in their reactions animal myosin and vitellin. They asserted

[1] Report of the Conn. Agricuitural Experiment Station, 1893, p. 186, and This Journal, 16, 633.

[2] Die Eiweisskörper, etc., Bonn., 1872. pp. 159, 176.

[3] Pflüger, Archiv., 16, 293, *1877*.

[4] Pflüger, Archiv., 18, 236, *1878*.

[5] *Physiol. Chemie.*, p. 75.

[6] *Ztschr. phys. Chem.*, 1, 72.

that the substance called legumin by Ritthausen was doubtless originally a globulin and that the preparations of this substance described and analyzed by him were altered by the alkali which he used in extracting them and were not the proteids originally contained in the seeds. Ritthausen contended strongly against this view and maintained that his preparations were wholly unaltered by the alkali. He extracted several kinds of seeds with salt solution, precipitated the proteid by dilution with water and found that the preparations of legumin so made were not essentially different in composition from those obtained by extracting with dilute potash water.[1] He then examined his older preparations, made by extracting the seeds with weak alkali and showed that they were to a very considerable extent soluble in salt solution. The substance thus extracted had, in many cases, a different composition from that of the original preparation, and Ritthausen then concluded that all the preparations which he had previously described as legumin were, in fact, mixtures of the two proteids, one, soluble in salt solution after dissolving in potash water and precipitating with acid, similar to, but distinct from conglutin, and the other originally soluble in salt solution but rendered insoluble in that fluid by treatment with alkalies. This latter he called legumin.

He then purified the legumin by extracting the mixed proteids from the seed with dilute alkali, neutralizing with acid, extracting the precipitate so produced with sodium chloride solution to remove proteids soluble in that fluid and then redissolving the residue, consisting mostly of legumin, in dilute alkali and reprecipitating with acetic acid.

Two preparations were so obtained, one from the pea and another from the horse bean (*Vicia faba*).

Ritthausen regarded his study of these preparations as showing that the substance from *Vicia faba* was a compound of tannic acid with the salt soluble proteid and that it was doubtful whether the horse bean contains legumin at all.

The preparation from the pea he finally considered to be legumin, having the following composition:

[1] *J. prakt. Chem.*, 26, 504.

LEGUMIN OF PEA, RITTHAUSEN.

Carbon	51.34
Hydrogen	6.98
Nitrogen	17.48
Sulphur	0.45
Oxygen	22.75
	100.00

This analysis represents the composition of legumin not in its original condition, but so altered as to be insoluble in saline solutions. Of the reactions of legumin we know little more than that it dissolves in salt solution and is precipitated by diluting with water.

In the following pages we give the outcome of our recent investigation into the composition and properties of legumin as contained in the seeds of the pea and the vetch.

Here, as in former papers, we have described our procedure with considerable, perhaps unnecessary, detail, but having often experienced great difficulty in understanding and repeating the work of our predecessors because of the vagueness of their statements, we have endeavored to describe our methods and results so fully and accurately that any who may wish to review our investigations experimentally may find it practicable to do so.

I. PROTEIDS OF THE PEA.

One hundred grams of garden peas ground to pass a sieve of one mm. mesh were extracted with petroleum naphtha to remove oil, then dried by exposure to the air, and finally treated with one liter of ten per cent. sodium chloride solution. As the very viscid extract could scarcely be filtered through paper, an equal volume of ten per cent. sodium chloride solution was added, and after some time one-half the solution passed the filter clear. This was saturated with ammonium sulphate, the resulting precipitate was filtered out, dissolved in salt solution, and the liquid dialyzed free from chlorides. The proteid separated, as do all vegetable globulins thus far observed, in spheroids. No distinct crystals could be detected in this or any of our preparations from the pea. When the chloride had been removed by dialysis the precipitate was filtered out, washed with water and alcohol, dried over sulphuric acid, and found to weigh three

and a half grams, being about seven per cent. of the meal. Dried at 110° this preparation was analyzed with the following results :

PEA LEGUMIN, 1.

Carbon	52.03
Hydrogen	6.96
Nitrogen	17.98
Sulphur, Oxygen	23.03
	100.00
Ash	0.41

Another preparation was made by extracting 500 grams of pea meal with three liters of ten per cent. sodium chloride brine and after allowing the mixture, protected with thymol, to stand three days in a cool place, 1500 cc. of the extract were decanted. Although very turbid, this was saturated with ammonium sulphate without filtering, and the precipitate produced was filtered out and dissolved in brine. The resulting solution was then filtered without much trouble and the clear filtrate dialyzed free from chlorides. After washing and drying the globulin thus precipitated, and amounting to ten grams or about five per cent. of the meal, had the following composition :

PEA LEGUMIN, 2.

	I.	II.	Average.
Carbon	52.08	52.19	52.14
Hydrogen	7.06	6.95	7.01
Nitrogen	18.01	17.91	17.96
Sulphur	0.49	....	0.49
Oxygen	....	....	22.40
			100.00
Ash	0.33		

In order to obtain larger quantities of this proteid for fractional precipitations 800 grams of pea meal were treated with four liters of *twenty* per cent. sodium chloride solution, and by draining on filters over night about one-half the solution applied to the meal, or two liters, was obtained as a clear yellow filtrate, which was saturated with ammonium sulphate, but for a reason, then unknown, very little proteid separated. Dilute acetic acid saturated with ammonium sulphate was then added in small

amount and the proteid separated as a flocculent precipitate. This was filtered out and in order to remove the acid as completely as possible the precipitate was suspended in about four liters of saturated ammonium sulphate solution and again filtered out. The precipitate was then dissolved in ten per cent. sodium chloride solution and calcium carbonate added to neutralize the acid retained by the proteid. The solution then reacted alkaline with litmus owing to ammonium carbonate set free from the sulphate. The solution was next filtered very nearly clear and dialyzed until a large precipitate had formed. This precipitate was filtered out, dissolved in salt solution, filtered clear and dialyzed free from chlorides. The precipitated globulin was washed with water and alcohol and dried over sulphuric acid, giving fifty-two grams, in whose analysis, after drying at 110°, the following figures were obtained:

PEA LEGUMIN, 3.

	I.	II.	Average.
Carbon	52.30	52.27	52.29
Hydrogen	7.06	6.98	7.02
Nitrogen	17.72	17.79	17.76
Sulphur	0.30	....	0.30
Oxygen	....	....	22.63
			100.00
Ash	0.53		

The solution filtered from this substance after its first precipitation by dialysis was saturated with ammonium sulphate, the resulting precipitate filtered out and dissolved in a little water, filtered clear and dialyzed. After removing the greater part of the salts by dialysis the precipitated globulin was filtered out, treated in the usual manner, and gave 14.2 grams of preparation 4, having, when dried at 110°, the following composition:

PEA PROTEID, 4.

	I.	II.	Average.
Carbon	52.50	....	52.50
Hydrogen	6.74	....	6.74
Nitrogen	16.83	16.76	16.80
Sulphur	0.49	....	0.49
Oxygen	....	....	23.73
			100.00
Ash	0.33		

Preparation 3, when dissolved in ten per cent. salt solution, was found to become turbid at 97° and after long heating in a boiling water-bath slowly developed a coagulum. Preparation 4 contained a considerable quantity of proteid coagulating at a much lower temperature. It was accordingly dissolved, as far as possible, in a little ten per cent. salt solution and the insoluble matter filtered out. The clear filtrate was diluted with distilled water until the solution contained 0.66 per cent. of salt, when a not inconsiderable precipitate formed, which was filtered out and the filtrate saturated with ammonium sulphate. This produced a relatively abundant precipitate, which was filtered out and dissolved in water. This solution, on heating, became turbid at 52°, and on keeping for some time at this temperature a minute quantity of flocks separated. Filtered at 56°, turbidity occurred again at 62° and a few flocks formed at 66°. Filtered at 67°, the solution became turbid at 70°, the turbidity increasing above 75° to a heavy flocculent coagulum at 79°.

It is evident from these results that we have in preparation 4 at least two proteids, one coagulating at 79°, the other being only slowly and imperfectly coagulated at 99°–100°; the former is readily soluble in very dilute salt solutions, the latter only slightly soluble in solutions containing less than one per cent. of salt. The filtrate from preparation 4 was dialyzed in water, but as no more globulin separated, the dialyzer was transferred to alcohol and the proteid thus completely thrown down. After washing with absolute alcohol and drying over sulphuric acid 12.31 grams of substance were obtained. This, of course, was a mixture of all the proteids extracted from the pea which had not been precipitated by dialysis in water. It was therefore treated with two per cent. salt solution, a large quantity of proteid which had been coagulated by the alcohol was filtered out, washed with water, with dilute and absolute alcohol, and dried over sulphuric acid. This preparation, 5, weighed 7.45 grams, and gave the following results when analyzed, after drying at 110°.

PEA PROTEID, 5.

	I.	II.	Average.
Carbon	53.40	53.26	53.33
Hydrogen	6.92	7.03	6.98
Nitrogen	16.19	16.09	16.14
Sulphur	1.00	....	1.00
Oxygen	....	....	22.55
			100.00
Ash			0.32

The filtrate from 5 was saturated with ammonium sulphate, whereupon a small gummy precipitate appeared which was filtered out and dissolved in a small quantity of water. This solution when heated became turbid at 49° and flocculent at 60°; filtered at 75°, turbidity occurred on heating again to 72° and flocks formed at 79°. After heating to about 90° no more proteid was coagulated by boiling. The solution now contained a very small quantity of proteose.

Since acetic acid was used to separate the substance, from which 3, 4 and 5 were obtained, from the ammonium sulphate solution, it was necessary to obtain more of the proteids without the use of acid. It was found that the incomplete precipitation by ammonium sulphate was due to the use of twenty per cent. sodium chloride solution, in which less ammonium sulphate dissolves than in a ten per cent, salt solution, not enough, in fact, to completely precipitate the proteid. The meal residue was therefore treated with water enough to reduce the strength of the salt solution still adhering to it to about ten per cent. A further considerable quantity of nearly clear extract was thus obtained, which, when saturated with ammonium sulphate, readily and completely parted with the proteid. This was filtered out, dissolved in ten per cent. brine, the solution filtered perfectly clear and dialyzed. After a large quantity of globulin had separated in the dialyzer its contents were filtered off, the precipitate was dissolved in ten per cent. salt solution and treated in exactly the same way as 3 had been. This preparation, 6, weighed 37.5 grams and, dried at 110°, had the following composition :

PEA LEGUMIN, 6.

	1.	II.	Average.
Carbon	52.37	....	52.37
Hydrogen	6.90	....	6.90
Nitrogen	17.95	17.95	17.95
Sulphur	0.39	....	0.39
Oxygen	....	....	22.39
			100.00
Ash	0.28		

The filtrate from the first precipitation, by dialysis, of this substance, when saturated with ammonium sulphate gave a precipitate which was dissolved in a little water and the resulting solution was filtered clear and dialyzed. After most of the salts were thus removed the separated globulin was filtered out, washed and dried, and gave 2.44 grams of preparation 7, having the following composition, when dried at 110°:

PEA PROTEID, 7.

	I.	II.	Average.
Carbon	52.09	52.02	52.06
Hydrogen	6.96	7.08	7.02
Nitrogen	16.75	16.57	16.66
Sulphur	0.55	....	0.55
Oxygen	....	....	23.71
			100.00
Ash	0.20		

This analysis is in fair accord with that of the similarly obtained preparation 4.

The filtrate from 7 was dialyzed into alcohol and then absolute alcohol was added to the solution until all the proteids separated. The precipitate thus produced was filtered out, washed with absolute alcohol, dried over sulphuric acid and found to weigh 7.1 grams. Since this preparation might be a mixture of any unprecipitated globulin, with albumin and proteose, if these were present, it was treated with water and the considerable quantity of proteid coagulated by alcohol was filtered out, washed thoroughly with water and then with absolute alcohol and dried over sulphuric acid. This gave 4.05 grams of preparation 8, which, when dried at 110°, had the following composition:

PEA PROTEID, 8.

	I.	II.	Average.
Carbon	53.60	53.47	53.54
Hydrogen	6.99	6.98	6.99
Nitrogen	16.72	16.65	16.69
Sulphur	1.01	....	1.01
Oxygen	....	....	21.77
			100.00
Ash	0.32		

The analysis of 8 agrees well with that of 5 and it is probable that these figures pretty nearly represent the composition of a second proteid (globulin or albumin) readily soluble in very dilute salt solutions.

Having thus found evidence of the presence of at least two proteids in the pea extract, one less soluble than the other in very dilute salt solutions, it became necessary to subject the less soluble and more abundant globulin to thorough fractioning in order to learn whether it was homogeneous or a mixture.

Twenty-five grams of 3 were therefore dissolved in 250 cc. of five per cent. sodium chloride solution, filtered clear and the filter washed with fifty cc. of the same salt solution. A portion of the preparation had, as is usually the case with vegetable globulins when dried, passed into an insoluble form. This insoluble matter when treated with salt solution gave a gummy residue, which was difficult to filter out. No estimate of the amount of this substance could be made.

The clear salt solution of the globulin was diluted with twice its volume of water, making 750 cc. of a 1.67 per cent. solution of sodium chloride. After standing over night the proteid which had precipitated on dilution was collected on a filter, washed with water and alcohol and dried over sulphuric acid. Preparation 9 was so obtained, weighing five and one tenth grams and having, when dried at 110°, the composition given below.

The solution filtered from this substance was treated with an equal volume of water making 1500 cc. of a brine containing 0.84 per cent. of salt, from which after standing some time a part of the proteid separated as a viscid layer at the bottom of the beaker. The solution was decanted and the precipitate

washed and dried in the usual manner. This, 10, weighed 5.29 grams. The decanted liquid was then dialyzed free from salt and the precipitated globulin treated in the usual manner, giving 11, weighing 4.10 grams. About three-fifths of the original substance was thus recovered in three nearly equal fractions. The other two-fifths consisted largely of insoluble globulin. The composition of the fractions so obtained was as follows :

PEA LEGUMIN, FRACTIONS OF 3.

	9.			10.			11.		
	I.	II.	Average.	I.	II.	Average.	I.	II.	Average.
Carbon	52.49	52.23	52.36	52.31	52.09	52.20	52.25	52.25	52.25
Hydrogen	7.11	7.10	7.11	7.09	6.92	7.01	7.08	....	7.08
Nitrogen	17.96	18.05	18.01	17.98	17.96	17,97	17.88	17.84	17.86
Sulphur	0.35	....	0.35	0.35	....	0.35	....	....	22.81
Oxygen	....	....	22.17		....	22.47	....	....	
			100.00			100.00			100.00
Ash			0.22			0.61			0.20

Again, twenty-five grams of preparation 6 were dissolved in 250 cc. of five per cent. brine, the solution filtered, the residue washed with fifty cc. of the same brine and the clear filtrate diluted with one and a half volumes of water, thus giving a two per cent. salt solution. After standing over night the precipitate was filtered out, washed with water and alcohol and dried over sulphuric acid. Preparation 12 so obtained weighed 8.58 grams.

The filtrate from 12, on adding an equal volume of water and treating the precipitate as just described, yielded 13, weighing 2.84 grams.

The filtrate from 13, dialyzed free from salt, gave 14, weighing four and two tenths grams.

PEA LEGUMIN, FRACTIONS OF 6.

	12.			13.		14.		
	I.	II.	Average.		I.	II.	Average.
Carbon	52.26	....	52.26	52.08	52.01	52.02	52.02
Hydrogen	6.96	....	6.96	7.04	....	7.20	7.20
Nitrogen	17.96	18.06	18.01	17.88	17.81	18.03	17.92
Sulphur	0.44	....	0.44	23.00	....	....	23.86
Oxygen	....	....	22.33		....	....	
			100.00	100.00			100.00
Ash			0.40	0.19			0.17

Comparing the analyses of these fractions with each other and with that of the original substance, it is plain that they all represent a single proteid.

SUMMARY OF ANALYSES OF PEA LEGUMIN.

	1.	2.	3.	6.	9.	10.	11.
Carbon	52.03	52.14	52.29	52.37	52.36	52.20	52.25
Hydrogen	6.96	7.01	7.02	6.90	7.11	7.01	7.08
Nitrogen	17.98	17.96	17.76	17.95	18.01	17.97	17.86
Sulphur	23.03	0.49	0.30	0.39	0.35	0.35	22.81
Oxygen		22.40	22.63	22.39	22.17	22.47	
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

	12.	13.	14.	Average.
Carbon	52.26	52.08	52.02	52.20
Hydrogen	6.96	7.04	7.20	7.03
Nitrogen	18.01	17.88	17.92	17.93
Sulphur	0.44	23.00	22.86	22.45
Oxygen	22.33			
	100.00	100.00	100.00	100.00

Ritthausen obtained from peas by extraction with salt solution and precipitation with water two preparations, the analyses of which are given below, A and B.[1]

By treating peas with very weak potash water, adding acid to neutralization, extracting the precipitate thus produced with salt solution and filtering out the insoluble matter, he obtained a solution from which, by adding water, a precipitate was thrown down whose composition is given below under C.

PEA LEGUMIN.

	Ritthausen.			Osborne & Campbell. Average of 18 analyses on 10 preparations.
	A.	B.	C.	
Carbon	52.83	51.61	51.62	52.20
Hydrogen	7.27	7.08	6.96	7.03
Nitrogen	17.26	17.23	18.26	17.90
Sulphur	22.64	24.08	0.33	0.39
Oxygen			22.83	22.48
	100.00	100.00	100.00	100.00

Ritthausen's preparation C agrees fairly well with the average of our results. The preparations extracted directly from peas

[1] *J. prakt. Chem.*, 26, 504.

by salt solution would appear to be the same substance but less pure. We see no ground for Ritthausen's idea that his older preparations were mixtures of two proteids both originally soluble in salt solution, one of which, legumin, is rendered insoluble in salt solution by treatment with alkalies. It is much more probable that a part of the globulin in his preparations had assumed the insoluble condition during the process of separating since nearly all globulins, to a greater or less extent, are prone to this change. The difference in composition between Ritthausen's original "legumin" and the substance extracted from it by salt solution is doubtless due to the greater purity of the latter. This view is supported by the close agreement in composition of this substance with those extracted by us directly from the pea. For this, the chief proteid of the pea, it is proper to retain the name *Legumin* first proposed by Braconnot.

The properties of legumin are as follows:

In water it is entirely insoluble.

In ten per cent. sodium chloride solution, when freshly prepared and not dried, it is readily soluble, but after washing with alcohol and drying over sulphuric acid, more or less becomes insoluble in salt solution. Dissolved in ten per cent. sodium chloride solution legumin is not precipitated by saturating the solution with magnesium sulphate, or sodium chloride. Saturated with sodium sulphate at 20°, no precipitate is produced; saturated at 25°, a turbidity appears; but saturated with sodium sulphate at 34°, all but a trace is thrown out of solution. By saturation with ammonium sulphate at common temperatures it is completely precipitated.

Dissolved in salt solution, legumin is not precipitated by mercuric chloride but gives a heavy precipitate on adding either picric, tannic, hydrochloric, nitric, sulphuric, or acetic acid.

In water containing a very small quantity of acid, legumin readily dissolves and is precipitated by adding sodium chloride. It is readily soluble in dilute alkalies and alkali carbonates.

Adding to its solution glacial acetic acid and concentrated sulphuric acid, a violet color is produced. With cupric sulphate and caustic potash, after standing, a crimson red color appears, almost as red as that given by peptones. With Mil-

lon's and the xantroprotein tests the usual reactions are given. When dissolved in ten per cent. sodium chloride solution and gradually heated, the solution becomes turbid at 97° and on long heating in a boiling water-bath, a coagulum gradually separates.

II. PROTEIDS OF THE VETCH.

One hundred grams of finely ground meal of the seed of the common vetch (*Vicia sativa*) were treated with water and the extract, after filtering clear, was saturated with ammonium sulphate. The small precipitate thereby produced was filtered out and dissolved in water; the resulting solution was filtered clear and dialyzed until free from chlorides. The globulin thus precipitated, after washing with water and with alcohol, weighed 1.04 grams. The meal residue was then treated repeatedly with ten per cent. sodium chloride solution and after filtering clear the extract was saturated with ammonium sulphate, the precipitated proteid filtered out and disssolved in brine. The resulting solution was filtered clear and dialyzed until free from chlorides. The globulin thus precipitated, when washed with water and alcohol, and dried over sulphuric acid, weighed five grams. When dried at 110° this preparation, 15, had the foling composition:

VETCH LEGUMIN, 15.

Carbon	52.45
Hydrogen	6.98
Nitrogen	18.04
Sulphur	0.50
Oxygen	22.03
	100.00
Ash	0.27

The meal residue was next treated with two-tenths per cent. potash water, the extract filtered clear and neutralized with very dilute hydrochloric acid; the precipitate thus produced was dissolved in two-tenths per cent. potash water, the clear solution was neutralized with dilute hydrochloric acid and the precipitated proteid washed with water and alcohol and dried. This preparation, 16, weighed four and four-tenths grams and gave the following results on analysis:

VETCH PROTEID, 16.

	I.	II.	Average.
Carbon	52.43	52.43	52.42
Hydrogen	7.13	7.02	7.07
Nitrogen	16.55	....	16.55
Sulphur, Oxygen	....	....	23.96
			100.00
Ash			0.74

Four kilograms of vetch meal were next treated with twelve liters of ten per cent. sodium chloride brine and the residue washed with the same solution. The extract and washings were partly cleared by subsidence, then saturated with ammonium sulphate. The precipitate so produced was dissolved in brine, but the resulting solution was very difficult to filter. The greater part of the suspended impurities was removed by passing the extract through a loose bed of filter paper pulp and the proteid was again separated by saturation with ammonium sulphate. This precipitate was dissolved in brine and the solution, kept cold, then filtered perfectly clear. This solution was dialyzed in two portions, D and E. After nearly freeing from chlorides, a large precipitate formed in each dialyzer, which was filtered out. That obtained from E was washed with water and with alcohol as long as any coloring matter was extracted, and was then dried over sulphuric acid giving 120 grams of a slightly pink powder, which will be designated F. That from D was redissolved in ten per cent. sodium chlorine brine, the solution filtered perfectly clear and dialyzed until free from chlorides. All but a trace of proteid was thus thrown down. The precipitate was washed thoroughly with water and with alcohol and dried over sulphuric acid, yielding preparation 17, which weighed ninety grams and was very slightly colored. After drying at 110°, this preparation had the following composition:

VETCH LEGUMIN, 17.

	I.	II.	Average.
Carbon	51.98	51.97	51.98
Hydrogen	6.94	6.89	6.92
Nitrogen	17.96	18.00	17.98
Sulphur	0.45	....	0.45
Oxygen	....	....	22.67
			100.00
Ash			0.20

The filtrates from the first dialysis of solutions D and E were separately saturated with ammonium sulphate, the precipitates obtained were dissolved in water and the solutions filtered and dialyzed. The precipitate from D, thrown down by dialysis, was redissolved in salt solution and again precipitated by dialysis. The two preparations of globulin thus obtained were washed with water and with alcohol and dried over sulphuric acid, that from D weighed 4.11 grams, forming preparation 18, and that from E gave preparation 19, weighing 7.67 grams. On analyzing these preparations, dried at 110°, the following results were obtained :

VETCH LEGUMIN.

	18.	19.
Carbon	52.21	52.18
Hydrogen	6.82	6.82
Nitrogen	17.99	17.99
Sulphur	0.37	0.36
Oxygen	22.61	22.65
	100.00	100.00
Ash	0.23	0.12

The filtrate from the first precipitation by dialysis of 18 was united with the filtrate from 19 and the heat coagulation point determined in a portion of the solution, in which ten per cent. of sodium chloride had been dissolved. This solution became turbid at 56° and flocks separated at 63° in considerable quantity. After heating to 70° for some time and filtering, turbidity occurred on heating to 71° and a flocculent coagulum formed at 73°, about the same in amount as at 63°. After heating to 78° the solution was filtered and again heated, the turbidity forming a third time at 79° and flocks at 83° in smaller quantity than before. This slow and incomplete coagulation does not necessarily indicate the presence of several coagulable proteids in the solution, for there is no temperature interval between the successive coagula, the temperature at which turbidity occurs and a flocculent coagulum develops being determined, after the first coagulum has formed, by the temperature at which the solution was filtered. Each time the solution becomes turbid at a temperature just above that to which it had been previously heated and a flocculent coagulum separates at three or four degrees higher.

The presence of salts has much influence on the coagulation point, for another portion of this same solution, to which no salt had been added, became turbid at ten degrees lower than the portion wherein ten per cent. of sodium chloride had been dissolved and gave the last flocculent coagulum at a temperature ten degrees higher.

The solution, portions of which had served for the foregoing observations, was then dialyzed into alcohol until concentrated to one-half its original volume, when a considerable precipitate formed, which was filtered out, washed with alcohol, and dried over sulphuric acid. This substance consisted of a mixture of all the proteids remaining in solution after separating the globulin, as described. Any albumin or globulin which might be contained in this precipitate would probably be largely if not wholly coagulated by the long treatment with alcohol and the subsequent drying. This preparation was therefore very finely pulverized and extracted thoroughly with water. The insoluble residue was then washed with alcohol and dried, yielding 13.52 grams of preparation 20, which was found to have the following composition :

VETCH PROTEID 20.

	I.	II.	Average.
Carbon	53.45	53.65	53.55
Hydrogen	6.67	6.73	6.70
Nitrogen	16.46	....	16.46
Sulphur	1.02	....	1.02
Oxygen	....	....	22.27
			100.00
Ash	0.29		

The solution filtered from 20 was further dialyzed into alcohol and a second precipitate obtained, which, when washed with alcohol and dried, weighed 5.64 grams, and was composed as follows :

VETCH PROTEID, 21.

	I.	II.	Average.
Carbon	52.55	52.66	52.60
Hydrogen	6.70	6.95	6.83
Nitrogen	16.53	16.76	16.69
Sulphur	1.23	....	1.23
Oxygen	....	....	22.65
			100.00
Ash	0.65		

The filtrate from the precipitate produced by the first dialysis into alcohol, from which preparations 20 and 21 had been obtained, was further dialyzed into alcohol yielding a second precipitate which, when washed with alcohol and dried over sulphuric acid, weighed 2.21 grams and formed preparation 22. This consisted of proteose and, after drying at 110°, had the composition as follows:

VETCH PROTEOSE, 22.

	I.	II.	Average.
Carbon	50.95	50.76	50.85
Hydrogen	6.78	6.72	6.75
Nitrogen	16.53	16.79	16.65
Sulphur, Oxygen	....	....	25.75
			100.00
Ash	2.18		

Comparing the composition of 21 with that of 20, it is seen that, excepting carbon, the figures agree quite well. 21, however, contains one per cent. less carbon than 20, which is easily explained by its being a mixture of the proteose represented by 22, and the proteid represented by 20. Such a mixture would be expected from the method of preparation.

If 20 is compared with 5 and 8, obtained in a similar manner from the pea, by dialysis of its extracts into alcohol, after precipitation of the greater part of the globulin contained in these extracts by dialysis in running water, it will be observed that they agree rather closely. It is hardly possible by this method to obtain entirely pure preparations, but our results show that the vetch and pea both contain another proteid that is different from legumin in composition and in properties.

To facilitate comparison these analyses are here tabulated.

	Pea proteid.		Vetch proteid.
	5.	8.	20.
Carbon	53.33	53.54	53.55
Hydrogen	6.93	6.99	6.70
Nitrogen	16.14	16.69	16.46
Sulphur	1.00	1.01	1.02
Oxygen	22.55	21.77	22.27
	100.00	100.00	100.00

It will be noted that this proteid contains more carbon and less nitrogen than legumin and nearly twice as much sulphur.

Whether it is a globulin soluble in extremely dilute salt solution or an albumin soluble in pure water, we have not as yet undertaken to ascertain, for want of time.

The residue of the meal extracted, as described, with salt solution, was treated with two-tenths per cent. potash solution, a portion of the alkali extract was filtered clear and neutralized with very dilute hydrochloric acid. The precipitate which resulted was dissolved in two-tenths per cent. potash water, and after filtering perfectly clear, again thrown down by neutralizing with hydrochloric acid. After drying 12.4 grams of 23 were obtained, having the following composition:

VETCH PROTEID, 23.

	I.	II.	Average.
Carbon	53.00	52.99	53.00
Hydrogen	6.91	7.02	6.97
Nitrogen	16.45	....	16.45
Sulphur	0.53	....	0.53
Oxygen	....	....	23.05
			100.00
Ash	0.92		

If this analysis is compared with that of 16, it will be noted that, although they agree in nitrogen content, they differ as respects carbon. The sulphur found in 23 would indicate that 23 is a mixture of legumin with other substances. It seems to us probable that it is mainly legumin which escaped extraction by the salt solution through imperfect pulverization of the meal or its incomplete exhaustion by the brine, or because it was present in the salt-insoluble form, a form which it may have assumed in the seed itself, or under the action of the solvents to which the meal was subjected. It has been our experience with other seeds that extractions with alkali, after exhausting the seed with salt solution, yields products which, in most cases, it is impossible to purify.

In order next to determine whether the legumin found in the vetch seed is a single proteid or a mixture, the following fractional precipitations were made.

One kilogram of the meal was extracted with ten per cent.

sodium chloride solution and, after filtering clear, the extract was saturated with ammonium sulphate and the proteids, thus precipitated, dissolved in 300 cc. of ten per cent. brine. The solution now measured 400 cc. and contained about eight per cent. of salt. After filtering perfectly clear, from a small amount of insoluble matter, an equal volume of distilled water was added. On standing a short time the proteid thus precipitated collected on the sides and bottom of the beaker as a sticky deposit, leaving the solution nearly clear. The latter was then decanted and the translucent, gummy mass of proteid washed with water, which caused it to turn opaque and become brittle, so that it was easily rubbed to a coarse powder.

After washing repeatedly with water the proteid was thoroughly washed with dilute alcohol, then with absolute alcohol, and dried over sulphuric acid. The preparation, 24, weighed 13.4 grams.

The solution decanted from 24 was cooled in an ice box over night and the clear supernatant liquid poured from the perfectly transparent semifluid layer which had thus formed on the bottom of the beaker. After washing and drying, 12.9 grams of preparation 25 were obtained. The solution decanted from 25 was mixed with an equal volume of distilled water and left over night in the ice box. A transparent layer of proteid was again deposited, which, when washed and dried, yielded 4.00 grams of 26.

The solution decanted from 26 was saturated with ammonium sulphate, the precipitated proteid dissolved in salt solution, and after filtering, the proteid was precipitated by dialysis. The globulin thus separated, after washing and drying, weighed 3.35 grams, and formed preparation 27.

The following figures were obtained by analyzing these preparations when dried at 110°.

VETCH LEGUMIN.

	24.	25.	26.	27.
Carbon	52.05	51.78	52.17	52.04
Hydrogen	6.99	6.89	6.92	7.06
Nitrogen	18.02	18.06	17.70	18.02
Sulphur	0.56	0.48	23.21	22.88
Oxygen	22.38	22.79		
	100.00	100.00	100.00	100.00

Several grams of preparation E, described on page 598, were dissolved in a little two-tenths per cent. potash water, the solution was diluted considerably with distilled water and carbon dioxide passed through it. At first the solution remained clear, but after a time the proteid suddenly and almost completely separated as a voluminous precipitate, the filtrate from which yielded but a trace of proteid on saturating with ammonium sulphate. The precipitate was washed with water and then treated with salt solution. A part dissolved and the rest was converted into a swollen gelatinous mass which rendered filtration impossible. After standing over night the solution was poured off and the gummy residue was washed by decantation, at first with salt solution and then with water. On washing out the salt, the residue lost its gummy character and became a dense, rapidly settling precipitate which was readily collected on a filter and completely washed with water and then with alcohol. After drying over sulphuric acid it furnished 2.62 grams of preparation 28. This peculiar behavior of legumin which has lost its solubility in salt solution, we have observed in a number of cases.

E, when treated directly with salt solution, behaved in exactly the same manner as the precipitate obtained by passing carbon dioxide through its solution in dilute potash water, that is, a part dissolved and a part remained as a gummy residue, which was dehydrated (?) by washing with water. The saline solution described above, which had been decanted from the part of the carbon dioxide precipitate which was insoluble in salt solution, was filtered clear and dialyzed free from chlorides. The precipitate which resulted was filtered out, washed and dried in the usual manner, and yielded 29. These two preparations were found to have the following composition:

VETCH LEGUMIN.

	28.	29.
Carbon	52.11	51.89
Hydrogen	6.82	6.88
Nitrogen	18.17	18.09
Sulphur	0.53	0.40
Oxygen	22.37	22.74
	100.00	100.00
Ash	0.27	0.13

Several grams of preparation 17 was dissolved in a little two-tenths per cent. potash water and the resulting clear solution was neutralized with dilute acetic acid, thereby precipitating the proteid. A portion of this precipitate was tested with ten per cent. sodium chloride solution and found to dissolve to a large extent and on warming to 50° nearly all went into solution. The remainder of the precipitate was washed, dried and analyzed with the following results:

VETCH LEGUMIN, 30.

Carbon	52.06
Hydrogen	6.80
Nitrogen	17.98
Sulphur	0.53
Oxygen	22.63
	100.00
Ash	0.15

Another portion of preparation 17 was dissolved in a little two-tenths per cent. hydrochloric acid and yielded a clear solution, which was neutralized with one-half per cent. sodium carbonate solution. The resulting precipitate was partly soluble in ten per cent. salt solution. It was washed and dried and, as preparation 31, gave the following figures when analyzed:

VETCH LEGUMIN, 31.

Carbon	52.12
Hydrogen	6.68
Nitrogen	18.20
Sulphur	0.40
Oxygen	22.60
	100.00
Ash	0.15

For convenience of comparison the analyses of legumin from the vetch are brought together in the following tables:

SUMMARY OF ANALYSES OF VETCH LEGUMIN.

	15.	16.	18.	19.	24.	25.
Carbon	52.45	51.98	52.21	52.18	52.05	51.78
Hydrogen	6.98	6.92	6.82	6.82	6.99	6.89
Nitrogen	18.04	17.98	17.99	17.99	18.02	18.06
Sulphur	0.50	0.45	0.37	0.36	0.56	0.48
Oxygen	22.03	22.67	22.61	22.65	22.38	22.79
	100 00	100.00	100.00	100.00	100.00	100.00

	26.	27.	28.	29.	30.	31.
Carbon	52.17	52.04	52.11	51.89	52.06	52.12
Hydrogen ...	6.92	7.06	6.82	6.88	6.80	6.68
Nitrogen	17.70	18.02	18.17	18.09	17.98	18.20
Sulphur . .	23.21	22.88	0.53	0.40	0.53	0.40
Oxygen....			22.37	22.74	22.63	22.60
	100.00	100.00	100.00	100.00	100.00	100.00

It will be seen from the following statement that the composition of legumin from the pea is identical with that from the vetch.

LEGUMIN.

	Pea. Average of 18 analyses on 10 preparations.	Vetch. Average of 13 analyses on 12 preparations.
Carbon	52.20	52.09
Hydrogen	7.03	6.88
Nitrogen.....	17.93	18.02
Sulphur	0.39	0.46
Oxygen.....	22.45	22.55
	100.00	100.00

What we have already stated concerning the properties and reactions of pea legumin applies strictly to that from the vetch except in two particulars. The solutions of pea legumin in ten per cent. brine when heated nearly to boiling become turbid and, after a time, a considerable coagulum separates in the form of a semi-solid clot. Similar solutions of the vetch legumin, on the other hand, remain perfectly clear, even after prolonged boiling.

Many carefully conducted experiments made with the legumin from each of these seeds, wherein the same quantity of globulin was dissolved in the same amount of salt solution of the same strength, were carried out side by side, but always with the same results, the pea legumin coagulating to a greater or less extent while the vetch legumin remained wholly unaffected.

That this difference is due to some foreign substance is indicated by the following experiment: A quantity of ten per cent. sodium chloride extract of pea meal was filtered clear and divided into two parts, one of which was dialyzed directly, the

other was saturated with sodium chloride and filtered clear. The latter solution was less viscid and much more easily filtered, presumably due to the removal of gum. This solution, saturated with salt, was then dialyzed.

The globulin precipitated by dialysis from each of the above named solutions, was dissolved in brine to new solutions containing ten per cent. of globulin and eight per cent. of sodium chloride. When these two solutions were heated, side by side, in the same water-bath and for the some length of time. a most marked difference was observed in the quantities of coagulum that appeared. Each solution contained a small quantity of the proteid coagulating at about 80°, so that after being heated to 85° for some time, they were filtered clear and again heated.

Each solution then became turbid at 93° and, after heating the bath to boiling for a little time, the solution of the globulin from the salt saturated extract became curdy, from the separation of a moderate quantity of coagulum, while that from the unsaturated extract set to a firm opaque jelly, so that the tube could be inverted without displacement of its contents.

The second difference noted was very slight, but appeared to be constant. By precipitating the legumin from the pea by dialysis, the proteid was obtained in the form of spheroids which showed little tendency to adhere in masses, while that from the vetch was always obtained in more or less coherent lumps which, however, were not at all fluid and gummy, but were easily broken up on stirring. In our opinion, the legumin from these two seeds is one and the same substance, or must, at least for the present, be so regarded.

SUMMARY.

1. So far as we have investigated, peas and vetches contain the same proteids, which are nearly if not entirely soluble in ten per cent. sodium chloride solution.

2. The greater part of these proteids consists of a globulin, the *Legumin* of Braconnot, which is readily precipitated by dialyzing its salt solutions.

The prevalent idea that legumin is soluble only in acids and alkalies is erroneous, it having been proved, notably by Ritt-

hausen, to be a true globulin. The composition of legumin, as shown by the average of our accordant analyses of thirty-one preparations obtained from the seeds of peas and vetches, is the following :

LEGUMIN.

Carbon	52.15
Hydrogen	6.96
Nitrogen	17.98
Sulphur	0.43
Oxygen	22.48
	100.00

Legumin is abundantly soluble in solutions containing above five per cent. of sodium chloride ; in those containing less salt it is not so soluble, the amount held in solution decreasing as the salt content diminishes, so that it is but sparingly soluble in solutions containing less than one per cent. of salt. By dilution with water, strong saline solutions of legumin are abundantly precipitated.

By saturation with sodium chloride or magnesium sulphate, its sodium chloride solutions are not precipitated ; by saturation with sodium sulphate at 25° they are not precipitated, but at higher temperatures more or less is thrown down, and by saturation with sodium sulphate at 34°, precipitation is very nearly complete. With nitric acid, Millon's and Adamkiewic's reagents it gives the usual proteid reactions.

With strong solutions of legumin the biuret test gives a violet color at first, which on standing becomes crimson red, similar to the color produced by peptones.

The legumin obtained by us from the vetch is not coagulated by heat nor even rendered turbid by prolonged boiling of strong solutions.

The legumin prepared by us from the pea is partly coagulated by heating strong solutions in a boiling water-bath, and sets to a firm jelly after thus heating for some time. These differences in their behavior on heating, and a greater tendency of the vetch legumin to cohere in semi-solid lumps when precipitated by dialysis, are the only points, of dissimilarity which a rigid comparison of preparations from the two seeds has revealed.

These differences, in our opinion, are due to the substances with which the proteid is associated in the two seeds, for saturation of the pea extracts with sodium chloride, before precipitating the legumin by dialysis, greatly diminished the amount of coagulum given by the pea legumin.

3. Besides the legumin, the pea and vetch contain another proteid in small amount, either an albumin or a globulin, soluble in extremely dilute salt solutions, and coagulated by heating its solutions to 80°. This substance we have not studied further than to make two preparations for analysis from the pea and one from the vetch. These were obtained in an insoluble form by coagulating with alcohol, so that the properties and reactions were not determined. The composition of this proteid is shown by the following average of three closely agreeing analyses :

PROTEID OF PEA AND VETCH.

Carbon	53.48
Hydrogen	6.89
Nitrogen	16.43
Sulphur	1.01
Oxygen	22.19
	100.00

4. In addition to the foregoing proteids a very little *proteose* was found in the extracts of both these seeds.

5. No attempt has yet been made to determine the total quantity of proteids in these seeds, nor to study minutely the proteids that occur in them in small proportion.

CONGLUTIN AND VITELLIN.[1]

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Received May 21, 1896.

REVIEW of the literature relating to the plant proteids hitherto described as conglutin and vitellin, shows that the subject is in great confusion, which can only be cleared up by a thorough examination of the seeds from which these proteids are said to have been obtained. This is the more important, because of late years various investigations have been made in

[1] From the Report of the Connecticut Agricultural Experiment Station for 1895. Communicated by the authors.