

leave the solution neutral to litmus, was made exactly like that described. This gave 3.75 per cent. of globulin on dialysis as against 4.35 per cent. extracted with water alone. By heating the filtrate from the above-mentioned preparation to 85° and washing and drying the coagulum, the amount of legumelin was found to be 2.03 per cent. of the meal.

### PROTEIDS OF THE LENTIL.<sup>1</sup>

By THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

Received March 29, 1898.

THE proteid substance of the lentil was first observed by Einhof in 1806<sup>2</sup>. Liebig<sup>3</sup> stated that plant-casein is obtained from beans, lentils, and peas. Dumas and Cahours extracted lentils with warm water, allowed the extract to deposit suspended impurities and precipitated the proteid from the decanted solution by adding acetic acid. After washing the substance thus separated with water and alcohol and drying it, they obtained the following figures by analysis :

Carbon .....	50.46
Hydrogen .....	6.65
Nitrogen .....	18.19
Oxygen, etc. ....	24.70
	<hr/>
	100.00

Ritthausen<sup>5</sup> described a single preparation of proteid from this seed, obtained in nearly the same way as the preceding, for which he gave the following composition :

Carbon .....	52.53
Hydrogen .....	6.84
Nitrogen .....	16.49
Sulphur .....	0.40
Oxygen .....	23.74
	<hr/>
	100.00

For our work, coarsely ground lentils were freed almost completely from the outer seed coats by a current of air and were then

<sup>1</sup> Reprinted from advance sheets of the Report of the Connecticut Agricultural Experiment Station for 1897. Communicated by the authors.

<sup>2</sup> Gehlen's *J. der Chem.*, 6, 543.

<sup>3</sup> *Ann. Chem. Pharm.*, 39, 138.

<sup>4</sup> *J. prakt. Chem.*, 28, 398.

<sup>5</sup> *Die Eiweisskörper*, etc., Bonn, 1872.

ground to a fine flour. Two kilograms of this flour were treated with ten liters of ten per cent. sodium chloride solution and after a short time the extract was strained out on fine bolting-cloth and allowed to stand over night in a cold place to deposit the suspended starch. The partly clarified extract was then passed through a centrifugal separator and finally filtered perfectly clear through a thick bed of filter-paper pulp. The extract was saturated with ammonium sulphate, the precipitate produced dissolved in dilute brine, the solution filtered perfectly clear and dialyzed for three days.

The globulin that had separated on dialysis was filtered out and, after washing with water and alcohol, dried over sulphuric acid. Preparation<sup>1</sup> 48 was thus obtained, which weighed 170 grams.

The solution from which 48 had separated was dialyzed for six days longer when it was filtered from a very small precipitate which, when washed and dried, formed preparation 49 that weighed 2.12 grams. The filtrate from 49 was saturated with ammonium sulphate, the separated proteid filtered out, dissolved in water, the solution filtered clear, and dialyzed. After prolonged dialysis but a trace of substance separated, which was filtered out, and the clear solution dialyzed in alcohol until all the proteid was precipitated. The substance thus obtained after drying weighed 6.78 grams. It was ground fine, exhausted with water and washed with alcohol, forming preparation 50.

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

	48 <sup>1</sup>	49	50
Carbon .....	51.59	52.12	53.31
Hydrogen .....	6.99	6.88	6.71
Nitrogen.....	17.63	16.21	16.08
Sulphur .....	0.56	0.79	0.97
Oxygen.....	23.23	24.00	22.93
	<hr style="width: 50px; margin-left: auto; margin-right: 0;"/>	<hr style="width: 50px; margin-left: auto; margin-right: 0;"/>	<hr style="width: 50px; margin-left: auto; margin-right: 0;"/>
	100.00	100.00	100.00
Ash .....	0.49	0.79	0.74
Amount .....	170.0 grams	2.12 grams	6.78 grams.

<sup>1</sup> Numbered consecutively with the preparations of the proteids of the pea. This Journal, 19, 494.

In order to subject 48 to fractional precipitation one hundred grams were treated with 250 cc. of water and 250 cc. of ten per cent. brine added. A large part failed to dissolve, having been converted into an insoluble form by the process of separation. This part was collected on filters, washed with cold five per cent. brine, and then with hot brine of the same strength, the cold and hot washings being kept separate. The residue was next washed with water and with alcohol and dried, giving 62.23 grams of preparation 51. The washings made with hot brine were diluted with four volumes of water so as to form a one per cent. salt solution and the precipitate produced allowed to settle, when the solution was decanted and the deposit washed with water and alcohol and dried, yielding preparation 52, weighing 3.28 grams.

The solution filtered from 51, which measured 300 cc., was mixed with the cold washings, and diluted until the solution contained two per cent. of salt. The rapidly settling precipitate which separated was washed with water and with alcohol, and when dried weighed 4.75 grams, preparation 53.

The solution decanted from 53 was treated with an equal volume of water making a one per cent. brine, whereby substance was separated which, when washed and dried in the usual way, gave 9.5 grams of preparation 54.

The filtrate from 54 was cooled during the night to about 5° which caused a further deposit, that by the usual treatment gave 7.69 grams of preparation 55.

So large a proportion of 48 had become insoluble in salt solution, that it was thought best to make another lot of the globulin, which if possible should be more soluble. Accordingly 1000 grams of lentil flour were extracted with ten per cent. brine and the globulin separated by dialysis in exactly the same way as employed in making 48. Instead, however, of washing the substance on the filter with alcohol it was removed from the paper, redissolved in five per cent. brine, and the filters washed with the same solution. The filtrate and washings measured 800 cc. As 600 cc. of five per cent. brine had been used this solution contained thirty grams of salt. To this was added one liter of water, so that the resulting mixture contained 1.66 per cent. of sodium chloride and had a temperature of 25°. The large pre-

cipitate that appeared, soon settled to a coherent layer on the bottom of the jar, so that the solution, very nearly clear, could be decanted almost completely. The deposit was thoroughly washed with water and with alcohol, and after drying weighed 21.43 grams, preparation 56.

The solution decanted from 56 was cooled over night to 5°. The proteid separated as a dense semitransparent deposit which on washing with water became opaque and white. It was then dehydrated with absolute alcohol and dried, giving 16.10 grams of preparation 57.

The filtrate from 57 was dialyzed for four days whereby chlorides were completely separated. A coherent layer of globulin, which was deposited on the bottom of the parchment bag, when washed and dried weighed 32.8 grams and formed preparation 58.

These preparations were dried to constant weight and analyzed with the following results :

	GLOBULIN.							
	51	52	53	54	55	56	57	58
Carbon .....	51.52	51.43	51.44	51.62	52.13	51.53	51.39	52.05
Hydrogen ....	6.96	6.91	6.96	7.01	7.19	6.86	6.98	7.02
Nitrogen .....	17.69	18.02	17.99	17.87	17.47	18.06	18.03	17.29
Sulphur .....	0.50	23.64	0.44	0.46	0.23	0.49	0.44	0.21
Oxygen.....	23.33		23.17	23.04	22.98	23.06	23.16	23.43
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Ash.....	0.94	0.61	1.43	0.33	0.29	0.61	0.14	0.08

In this seed, as in the pea, we thus found that by fractional precipitation, the globulin is separated into two parts which differ in composition and solubility. As however none of the less soluble fractions was wholly free from coagulable matter, another extraction was made in which the acid of the seed was neutralized and the fractional precipitations were repeated until products wholly free from coagulable proteid were obtained.

Accordingly 2000 grams of meal were treated with ten liters of ten per cent. salt solution in which had been dissolved sufficient potash to yield an extract neutral to litmus, as determined by a preliminary trial. After thoroughly mixing, the whole was allowed to stand over night, with thymol, in a cool place, when the partly cleared extract was siphoned off and filtered. The solution was then saturated with ammonium sulphate, the pre-

precipitate filtered out, suspended in water and dialyzed over night. The proteid was thus dissolved, the solution filtered perfectly clear and dialyzed in two parts. Part I, which was obtained first, was treated as follows :

After dialyzing four days the precipitated globulin was filtered out and the filtrate A treated as subsequently described. The precipitate was dissolved in 500 cc. of two per cent. salt solution and 500 cc. of water added. A large precipitate formed, from which, after settling, the solution B was decanted.

The precipitate was then dissolved in 400 cc. of two per cent. salt solution and precipitated again by adding 400 cc. of water. The solution C was decanted from the precipitate thus thrown down, and the latter dissolved in five per cent. salt solution, and found to yield a considerable coagulum on heating in a boiling water-bath, showing it to still contain some vicilin. This latter solution was filtered clear and dialyzed for three days, when the precipitate which had formed was dissolved in 300 cc. of two per cent. salt solution, and 100 cc. of water added, causing a large precipitate which was found to be entirely free from coagulable matter. After washing this with water and alcohol, and drying over sulphuric acid, 23.67 grams of preparation 59 were obtained having the following composition when dried at 110° :

## LEGUMIN 59.

Carbon .....	51.80
Hydrogen .....	6.86
Nitrogen .....	18.09
Sulphur .....	0.42
Oxygen .....	22.83
	100.00
Ash .....	0.29

The solution decanted from 59 was treated with 200 cc. of water and the precipitate produced allowed to settle. The solution was then decanted and the precipitate washed with water and alcohol giving 5.36 grams of preparation 60, which, when dried at 110° and analyzed, gave the following results :

GLOBULIN, 60.

Carbon .....	51.83
Hydrogen .....	7.01
Nitrogen .....	17.75
Sulphur .....	0.29
Oxygen .....	23.12
	<hr/>
	100.00
Ash .....	0.46

The solution decanted from 60 was dialyzed free from chlorides and the precipitate thereby separated, after washing and drying as usual, gave preparation 61, which analysis showed to contain :

VICILIN, 61.

Carbon .....	52.13
Hydrogen .....	6.95
Nitrogen .....	17.40
Sulphur.....	0.22
Oxygen .....	23.30
	<hr/>
	100.00
Ash .....	0.21

Solution B, page 366, was diluted with an equal volume of water which caused a precipitate, from which, after settling, the solution was decanted. This was washed, dried, and analyzed with the following results :

VICILIN, 62.

Carbon .....	52.28
Hydrogen .....	7.02
Nitrogen .....	17.41
Sulphur.....	0.08
Oxygen .....	23.21
	<hr/>
	100.00
Ash .....	0.21

The filtrate from this preparation was saturated with ammonium sulphate, but very little proteid was found in it.

Solution C, page 366, was also treated with an equal volume of water which precipitated a further quantity of globulin, that, when dried, weighed 5.55 grams, and had the following composition :

## VICILIN, 63.

Carbon .....	52.14
Hydrogen .....	7.02
Nitrogen .....	17.27
Sulphur .....	0.18
Oxygen .....	23.39
	<hr/>
	100.00
Ash .....	0.21

Solution A, described on page 366, from which the greater part of the globulin extracted from the meal, had been removed by dialysis, was saturated with ammonium sulphate. The precipitate produced was suspended in water and dialyzed over night, thereby bringing the proteid into solution in a comparatively small volume of water. This solution was then filtered perfectly clear, and dialyzed forty hours, when a considerable precipitate separated which was filtered out, washed, dried, and analyzed with results as follows :

## VICILIN, 64.

Carbon .....	52.03
Hydrogen .....	6.91
Nitrogen .....	17.60
Sulphur .....	0.13
Oxygen .....	23.33
	<hr/>
	100.00
Ash .....	0.26

The filtrate from 64, after uniting with the similar solution from part II of this extract, was further dialyzed for nine days, until free from sulphates, when the precipitate which had formed was filtered out, washed, dried, and analyzed; It weighed only 1.13 grams and had the following composition :

## LEGUMELIN, 65.

Carbon .....	53.02
Hydrogen .....	—
Nitrogen .....	16.36
Sulphur } .....	—
Oxygen } .....	—
Ash .....	0.47

The clear solution filtered from 65 was then heated in a water-bath for three hours at 65° and the resulting coagulum filtered

out and the filtrate further heated to 82°, whereby a second coagulum was produced. These two preparations, 66 and 67 respectively, were washed with hot water and with absolute alcohol and gave on analysis, when dried at 110°, the following results :

LEGUMELIN.		
	66	67
Carbon .....	53.33	53.23
Hydrogen .....	6.87	6.88
Nitrogen.....	16.28	16.35
Sulphur.....	0.85	1.00
Oxygen.....	22.67	22.54
	100.00	100.00
Ash .....	0.20	0.41

The filtrate from 67 was concentrated by dialysis in alcohol and the proteid so precipitated filtered out, washed with absolute alcohol, and redissolved in a little water. This solution was dialyzed and found wholly free from globulin, and also from coagulable proteid. The solution was then precipitated by pouring into much alcohol and the substance so separated dried and analyzed.

PROTEOSE, 68.	
Carbon .....	50.17
Hydrogen.....	6.77
Nitrogen.....	16.81
Sulphur.....	1.27
Oxygen .....	24.98
	100.00
Ash .....	1.03

As already stated, the solution of the first ammonium sulphate precipitate of the proteids contained in the original extract was divided into two parts, which were dialyzed separately. The precipitate so produced in part II was dissolved in 500 cc. of two per cent. brine and 750 cc. of water added, giving an abundant precipitate, which was washed with water and alcohol and dried over sulphuric acid. This preparation, 69, wholly free from coagulable proteid, weighed nineteen grams and had, when dried at 110°, the following composition :



## LEGUMIN, 69.

Carbon.....	51.91
Hydrogen .....	7.11
Nitrogen .....	17.91
Sulphur.....	0.38
Oxygen .....	22.69
	<hr/>
	100.00
Ash .....	0.62

In order to further fractionate preparations 59 and 69, quantities of each were mixed together, dissolved in five per cent. salt solution and filtered from a very small amount of insoluble matter. That any acid possibly present in combination with this globulin might be neutralized, three-tenths per cent. potash solution was cautiously added until the solution reacted just perceptibly alkaline with litmus paper. This solution was then dialyzed over night and the precipitate which separated was filtered out and the filtrate dialyzed twenty-four hours longer, giving a second precipitate, which was washed and dried as usual, forming preparation 70, while the first dialytic precipitate was redissolved in ten per cent. brine, filtered perfectly clear and again dialyzed over night. The precipitate which separated was prepared for analysis in the usual manner and formed preparation 71. These two products were found to be free from coagulable matter and to have the following composition, which is essentially the same as that of the preparations from which they originated:

## LEGUMIN.

	70	71
Carbon .....	51.85	51.74
Hydrogen .....	6.88	6.87
Nitrogen .....	18.07	18.09
Sulphur .....	0.37	0.39
Oxygen.....	22.83	22.91
	<hr/>	<hr/>
	100.00	100.00
Ash .....	0.56	0.44

Since legumin has been described as soluble in water, 400 grams of lentil meal were twice treated with two liters of water, and strained on bolting-cloth. The extract was allowed to settle over night, and the somewhat turbid liquid (four liters) was siphoned off and saturated with ammonium sulphate. The precipitated proteids were treated with dilute brine, in which nearly

all dissolved. The solution was filtered clear and dialyzed for forty hours; the resulting precipitate, weighing, when dried, seven grams, was analyzed with the following results :

LEGUMIN, 72.	
Carbon.....	51.65
Hydrogen.....	6.90
Nitrogen.....	18.05
Sulphur.....	0.38
Oxygen .....	23.02
	100.00
Ash .....	0.64

One more extraction was made in the following manner : To 1200 grams of lentil meal, six liters of water were added which held in solution, 6.21 grams of baryta, the amount which had previously been determined to yield an extract neutral to litmus. After thoroughly mixing with the meal and breaking up all the lumps, six liters of ten per cent. salt solution were added. Unlike extracts not neutralized or neutralized with soda or potash, the insoluble matter in this case formed large flocculent masses which rapidly settled. The insoluble matter was strained out on bolting-cloth and the nearly clear extract was saturated with ammonium sulphate. The proteid thus separated was filtered out, dissolved in water, and dialyzed for three days. The greater part of the globulin was thus separated. It was filtered out and the filtrate was treated as described on page 372. The precipitate was dissolved in two per cent. brine and diluted until the solution contained 1.25 per cent. of salt. After depositing the resulting precipitate the solution was decanted and the precipitate was five successive times redissolved in fifty cc. of ten per cent. brine and thrown down by diluting to 400 cc. with water. The final precipitate, which was free from coagulable globulin, was washed with water and alcohol and dried. It weighed 15.85 grams and had the following composition :

LEGUMIN, 73.	
Carbon .....	51.63
Hydrogen .....	6.95
Nitrogen .....	18.00
Sulphur.....	0.43
Oxygen .....	22.99
	100.00
Ash .....	0.33

The solutions, resulting from the five just mentioned precipitations, and containing about one per cent. of salt, were diluted with an equal volume of water, forming a one-half per cent. salt solution. Proteid was thus precipitated which when dry weighed 20.25 grams, but was not further examined as it was doubtless a mixture of legumin and vicilin. The solution decanted from the foregoing precipitate was dialyzed for four days whereby 9.35 grams of preparation 74 were obtained having the following composition :

## VICILIN, 74.

Carbon .....	52.15
Hydrogen .....	6.81
Nitrogen .....	17.21
Sulphur.....	0.14
Oxygen .....	23.69
	<hr/>
	100.00
Ash .....	0.26

The filtrate described on page 371 from which the greater part of the globulin present in the extract had been separated by dialysis, was saturated with ammonium sulphate, the precipitate produced dissolved in a little water, and the solution filtered clear and dialyzed for seven days, when it was filtered from a small precipitate which analysis showed to be probably a mixture of vicilin and legumelin. The solution was returned to the dialyzer and the dialysis continued several days but nothing more separated. The solution was then concentrated by dialysis in alcohol and the precipitated proteid was dehydrated with absolute alcohol and dried. This preparation was then extracted with water, the residue remaining washed with alcohol, dried, and analyzed :

## LEGUMELIN, 75.

Carbon .....	53.10
Hydrogen .....	6.91
Nitrogen .....	16.16
Sulphur.....	1.09
Oxygen .....	22.74
	<hr/>
	100.00
Ash .....	0.56



## PROTEIDS OF THE LENTIL.

	LEGUMELIN.					
	50	65	66	67	75	Average.
Carbon . . . .	53.31	53.02	53.33	53.23	53.10	53.20
Hydrogen . . .	6.71	...	6.87	6.88	6.91	6.82
Nitrogen . . .	16.08	16.36	16.28	16.35	16.16	16.25
Sulphur . . . .	0.97	...	0.85	1.00	1.09	0.98
Oxygen . . . .	22.93	...	22.67	22.54	22.74	22.75
	-----	-----	-----	-----	-----	-----
	100.00	.....	100.00	100.00	100.00	100.00

## PROTEOSE, 68.

Carbon .....	50.17
Hydrogen .....	6.77
Nitrogen .....	16.81
Sulphur.....	1.27
Oxygen .....	24.98
	-----
	100.00

The quantity of proteid extracted by water and precipitated by dialysis was determined for the lentil, as follows :

To 200 grams of fine ground meal one liter of water was added; the mixture was poured upon a sieve and the lumps broken up by washing them through with another liter of water. After mixing and standing a short time, the coarse residue was strained out on bolting-cloth, and the fine suspended matter allowed to settle over night at a low temperature. The solution was then siphoned off, filtered perfectly clear on a pulp filter, the first 300 cc. containing the water retained in the pulp being rejected, and one liter of the clear undiluted extract next passing the filter dialyzed as long as anything precipitated. In this way 9.76 per cent. of proteid was recovered, which contained 17.32 per cent. of nitrogen, showing the substance to be nearly pure globulin.

This operation was repeated with the same quantities and proportions of materials, but with the addition of just enough baryta to the water to give an extract perfectly neutral to litmus. On dialysis 13.72 per cent. of globulin was obtained, a considerably larger quantity than that yielded by the unneutralized extract. Although the globulins extracted from the lentil by brine are legumin and vicilin, identical in composition and properties with those from the pea, yet the proportion extracted by water from

the lentil is much greater, especially when the acid of the seed is neutralized to litmus.

A rigid comparison of the reactions given by the aqueous extracts of the lentil, both acid and neutral, showed no difference whatever, and in these reactions the extracts agreed strictly with those similarly obtained from the pea, except that with calcium chloride and sulphate heavy precipitates were obtained, readily soluble in a slight excess of calcium or sodium chloride. Extracts of the pea gave only slight precipitates with calcium chloride and none with calcium sulphate.

---

### ON THE SPEED OF COAGULATION OF COLLOID SOLUTIONS.

BY C. E. LINEBARGER.

Received April 2, 1898.

ONE of the prettiest of projection experiments is the exhibition of the formation of crystals of salts, as ammonium chloride and oxalate, etc. The crystals seem fairly to shoot out over the screen. This speed of crystallization, so strikingly shown by the lantern, appears to be a characteristic property of solutions varying with the nature of the salt and solvent, the temperature, the degree of supersaturation, etc. So far as I know, no careful quantitative work has ever been done on the subject; some rough determinations that I have made with solutions of potassium nitrate indicate that the speed of crystallization is one or two millimeters per second.

Although there is a dearth of data on the subject, it will probably be conceded that the speed with which crystalloidal substances separate out of solution, is comparatively rapid; that is to say, the crystallizing solid traverses appreciable distances in a second or so.

Crystallization does not supervene unless the solution be supersaturated with respect to the solid, and there be at least a trace of a crystal already present to induce the crystallization; a supersaturated solution is then in an unstable condition.

Solutions of many colloids can be made to coagulate or gelatinize by the addition of a mere trace of certain substances. The striking analogy between this phenomenon and that of crystallization has often been mentioned and insisted