the stop-cocks in which it is used do not become set even after long standing. The last property makes it especially valuable for the Orsat apparatus.

ROSE POLYTECHNIC INSTITUTE.

PROTEIDS OF THE PEA.[1]

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

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IN a paper on the "Proteids of the Pea and Vetch"[2] the legumin obtained from these seeds was described and some account was also given of other accompanying proteids. Further study has furnished much additional information concerning these substances and made necessary a review of our former work on the pea and vetch.

On investigating the proteid constituents of the horse bean and lentil we found that by repeated fractional precipitations, the globulin from these seeds was separated into fractions, on the one hand wholly free from coagulable matter and on the other consisting of substance which was substantially all coagulable. The former we found to be legumin, in all respects like that described by us from the vetch, and the latter to be a new proteid of different composition and properties, to which we have given the name *vicilin* since we first recognized its presence in the horse bean (*vicia faba*).

This discovery led us to reinvestigate the proteids of the pea and we thus found that the legumin of that seed, as formerly described by us, was contaminated with more or less vicilin and that when the latter is completely separated the differences noted in our former paper between the legumin of the pea and that of the vetch disappear and preparations from these two seeds are identical in composition and reactions.

Leguminous seeds contain about one and two-tenths per cent. of alkali and one per cent. of phosphoric acid, while in the seeds of the cereals but five-tenths and seven-tenths per cent. of these substances respectively are present.

Liebig and Rochleder as well as Ritthausen attributed the

[1] Reprinted from advance sheets of the report of the Connecticut Agricultural Experiment Station for 1897. Communicated by the authors.

[2] This Journal, 18, 583; Report of the Connecticut Agricultural Experiment Station for 1895.

presence of much legumin in the aqueous extracts of leguminous seeds to the basic alkali phosphates. But the water extracts are strongly acid to litmus and accordingly must contain acid phosphates.

We find that a mixture of hydrogen potassium phosphate with enough phosphoric acid to be distinctly acid to litmus, but strongly alkaline to lacmoid, freely dissolves legumin to solutions that behave in all respects like the aqueous extracts of these seeds. Such solutions are unlike those obtained with neutral salts, not being easily precipitated by dilution unless holding a very large proportion of proteid. They yield precipitates with acetic acid which are wholly soluble in sodium chloride brine, thus resembling solutions in sodium bicarbonate.

In this and the following papers we give the results of our investigation upon each seed, and in the last paper, a general summary of the properties and composition of the different proteids which we have found.

Finely ground meal of garden peas was prepared in the laboratory almost entirely free from the outer coating of the seed. Immediately after grinding, 1,800 grams were treated with a large quantity of ten per cent. salt solution, strained through fine bolting-cloth and, after standing three hours to deposit insoluble matter, the turbid extract was siphoned off and saturated with ammonium sulphate. The precipitate was filtered out, suspended in a little water and dialyzed for eighteen hours. A large part of the ammonium sulphate was thus removed and the proteid dissolved by the dilute saline solution which remained. This solution was filtered perfectly clear through a thick felt of paper pulp and dialyzed for four days. A large precipitate resulted from which, after settling, the solution, A was decanted and treated as described on page 353 ; the precipitate was dissolved in about 1500 cc. of ten per cent. brine, the solution filtered clear and made exactly neutral to litmus paper by the cautious addition of seventy-five cc. of two-tenths per cent. potash water. The solution was then dialyzed for forty hours, whereby globulin separated out in well-defined spheroids which, after settling, united to a coherent mass exactly like vetch legumin, and differing from our previously described pea legumin that had formerly been obtained as a pulverulent pre-

cipitate. A portion of this precipitate was washed thoroughly with water and with alcohol and dried over sulphuric acid, giving 16.83 grams of preparation 32, which had, after drying at 110°, the following composition:

GLOBULIN, 32.[1]

Carbon	51.87
Hydrogen	7.01
Nitrogen	17.69
Sulphur	0.40
Oxygen	23.03
	100.00
Ash	0.62

This preparation, unlike those made from unneutralized solutions, was almost free from substances insoluble in salt solution. Dissolved in ten per cent. brine and heated above 90°, turbidity was produced, which at 98°–100° developed slowly into a considerable coagulum.

During the washing of this preparation considerable substance dissolved in water, yielding a solution which was perfectly neutral to litmus (indicating the absence of acid globulin), and which on adding a little salt, gave a precipitate that with more salt dissolved completely to a solution coagulable by heating.

The remainder of the proteid, of which 32 was a part, was dissolved by adding fifty cc. of ten per cent. salt solution and the resulting liquid was diluted with water until it contained about 1.25 per cent. of salt. A large part of the proteid was thus thrown down and settled out as a viscid layer, from which after an hour the supernatant fluid was almost completely decanted. This deposit, X, was then washed with 200 cc. of water, which caused it to become opaque and pasty. The wash-water, after decanting, was added to the solution from which the proteid had separated, and thereupon another precipitate, Y, resulted, from which, after settling, the solution was decanted and dialyzed, thereby yielding precipitate Z.

As precipitate X was found to give a decided turbidity on heating its solution in a boiling water-bath, it was dissolved by add-

[1] To avoid confusion and facilitate reference the preparations described in this paper are numbered consecutively with those given in the paper on "Legumin and other Proteids of the Pea and Vetch," this Journal, 18, 583.

ing ten grams of sodium chloride and water enough to make a final volume of 350 cc. The clear solution which resulted, containing about three per cent. of salt, was diluted with water until it contained about one and three-fourths per cent. of salt. A large precipitate, I, separated, which rapidly settled as a viscid, semi-fluid layer from which the solution II was soon completely decanted. As the precipitate I, when dissolved in salt solution, still gave a slight turbidity on heating to 99°, it was washed with water, dissolved in 100 cc. of ten per cent. salt solution, and reprecipitated by diluting the resulting clear liquid with 900 cc. of water. The precipitate which separated was now found to be wholly free from any coagulable matter, for not the slightest turbidity was produced in its solutions in brine even by prolonged heating in a boiling water-bath. The substance was therefore washed thoroughly with water and with alcohol and dried over sulphuric acid, thus giving 12.33 grams of preparation 33, which had, when dried at 110°, the following composition:

LEGUMIN, 33.

Carbon	51.74
Hydrogen	6.90
Nitrogen	18.04
Sulphur	0.42
Oxygen	22.90
	100.00
Ash	0.78

The solution decanted from 33 was dialyzed free from chlorides and a precipitate obtained which when washed and dried in the usual manner weighed 4.44 grams and had the composition shown by the following figures:

GLOBULIN, 34.

Carbon	52.06
Hydrogen	6.99
Nitrogen	17.86
Sulphur	0.36
Oxygen	22.73
	100.00
Ash	0.32

Solution II, page 351, was next diluted with an equal volume of water until it contained about nine-tenths per cent. of salt; after standing a short time the solution was decanted and the liquid precipitate washed thoroughly with water and alcohol and dried, giving 6.47 grams of preparation 35.

GLOBULIN, 35.

Carbon	51.95
Hydrogen	6.96
Nitrogen	17.76
Sulphur	0.42
Oxygen	22.91
	100.00
Ash	0.57

The filtrate from 35 was dialyzed free from chlorides and yielded 5.18 grams of preparation 36, which after the usual treatment gave on analysis the following results:

GLOBULIN, 36.

Carbon	52.43
Hydrogen	7.04
Nitrogen	17.45
Sulphur	0.23
Oxygen	22.85
	100.00
Ash	0.40

Preparations Y and Z obtained as successive precipitates from the solution decanted from X, as described on page 350, were separately washed with water and alcohol and dried over sulphuric acid, giving respectively preparation 37, weighing 8.06 grams, and preparation 38, weighing 6.80 grams. These had the composition given below:

GLOBULIN.

	37	38
Carbon	52.08	52.20
Hydrogen	6.90	7.01
Nitrogen	17.65	17.53
Sulphur	0.28	0.15
Oxygen	23.09	23.11
	100.00	100.00
Ash	0.75	0.46

The solution, described on page 349, that had been decanted from the mixed globulin and after forty hours' dialysis had yielded the preparations 32–38, was further dialyzed for four days and 11.2 grams of globulin obtained having, when dried at 110°, the following composition:

GLOBULIN, 39.

Carbon	52.45
Hydrogen	7.04
Nitrogen	17.22
Sulphur	0.16
Oxygen	23.13
	100.00
Ash	0.18

It will be noticed that by this extensive fractional precipitation of the pea globulin, a separation was effected which yielded legumin, agreeing in reactions with that of the vetch as formerly described, and with that of the lentil and horse bean, as will be shown later in this paper, and vicilin, a more soluble globulin of somewhat different composition, which is coagulated by heating its solutions to about 95° and has a very remarkably low content of sulphur. The composition of vicilin is shown by the analyses 36, 38 and 39, which represent the most soluble globulin contained in the solutions from which they were separated.

VICILIN.

	36	38	39
Carbon	52.43	52.20	52.45
Hydrogen	7.04	7.01	7.04
Nitrogen	17.45	17.53	17.22
Sulphur	0.23	0.15	0.16
Oxygen	22.85	23.11	23.13
	100.00	100.00	100.00

The reactions of legumin and vicilin will be given fully in a following paper after describing the preparation of these proteids from other legumes.

On page 349 we described how, after dialyzing for four days, the solution of the precipitate obtained by saturating the seed extract with ammonium sulphate, the greater part of the glob-

ulin was precipitated, and stated that the solution filtered from this globulin, there marked A, was reserved for further notice. This solution, which by dialysis had become bulky, was saturated with ammonium sulphate and the precipitate produced, filtered out, suspended in a little water and dialyzed over night. In this way the proteids were concentrated in a small volume of liquid. This solution was then filtered clear and dialyzed. After five days the precipitate which had separated was filtered out and extracted with ten per cent. salt solution, but as only very little dissolved, it was washed free from chlorides with water, then with alcohol and dried at 110°. Analysis showed it to have the following composition:

LEGUMELIN, 40.

Carbon	52.93
Hydrogen	7.10
Nitrogen	16.18
Sulphur	0 85
Oxygen	22.94
	100.00
Ash	0.22

The filtrate from 40 was then saturated with ammonium sulphate and the precipitate produced, dissolved in as small a volume of water as possible, filtered clear and dialyzed seven days. A very slight precipitate separated which was filtered out, washed thoroughly with water and found to dissolve in ten per cent. brine, giving a solution which yielded a flocculent coagulum at 53°; the heat coagulation point of the body we designate *legumelin*. The solution from which this small precipitate had separated, after filtering, was heated in a water-bath to 67° and a large coagulum produced which was washed thoroughly with hot water and then with alcohol and dried over sulphuric acid, giving eighteen grams of 41.

The filtrate from 41 was heated to 95° and the resulting coagulum, washed and dried, formed preparation 42, weighing 4.10 grams. Dried at 110° these gave the following results when analyzed:

LEGUMELIN.

	41	42
Carbon	53.24	53.36
Hydrogen	6.99	6.98
Nitrogen	16.15	16.24
Sulphur	1.09	1.01
Oxygen	22.53	22.41
	100.00	100.00
Ash	0.21	0.21

Another extract, obtained in substantially the same manner as that just described, after removing most of the globulin by dialysis, was saturated with ammonium sulphate and the precipitate produced was dissolved in a little water and its solution dialyzed for seven days, giving a precipitate which was filtered out and found to consist almost wholly of proteid matter insoluble in salt solution. After extracting with brine, this substance was thoroughly washed with water and dried, giving 4.24 grams of preparation 43. The filtrate from 43 was dialyzed in pipe water for five days longer, but as no more precipitate resulted, it was dialyzed for five days in a large volume of distilled water, which was frequently changed and kept cool by adding ice containing only an extremely small quantity of mineral matter. No precipitate was thus separated, and to the solution was then added ten per cent. of sodium chloride and acetic acid as long as a precipitate formed. The resulting precipitate was filtered out, dissolved in water and sodium carbonate added to very slight alkaline reaction. This caused a large precipitate which when prepared for analysis weighed 5.73 grams, preparation 44. The solution filtered from this neutralization precipitate, contained only a very little proteid, as shown by saturating the filtrate with ammonium sulphate.

The filtrate from the precipitate produced by acetic acid in the ten per cent. salt solution was unchanged on further addition of acetic acid and was therefore saturated with sodium chloride which gave a second precipitate. This was filtered out, dissolved in water, the solution filtered clear and dialyzed. After chlorides had diffused away the solution was perfectly neutral to litmus and quite clear. It was accordingly concentrated by dialysis in alcohol and the precipitated proteid, 45, dehydrated

by absolute alcohol, and dried, weighed 5.87 grams. These preparations were analyzed with the following results :

LEGUMELIN.

	43	44	45
Carbon	53.03	53.43	53.23
Hydrogen	7.10	7.01	6.91
Nitrogen	16.30	16.50	16.08
Sulphur	1.01	1.16	1.11
Oxygen	22.56	21.90	22.67
	100.00	100.00	100.00
Ash	0.35	0.80	0.56

It will soon be shown that the proteose of the pea is precipitated by acetic acid from a solution saturated with sodium chloride, and it might be expected that 45 would therefore contain a large proportion of proteose; but as it was found that this preparation through washing with alcohol and drying at 110° had become insoluble in water, it is probable that the proteose had mostly diffused away during the long dialysis to which the solutions had been subjected.

If the last six analyses are compared, it will be seen that they are in close agreement except 40, in which carbon and sulphur are somewhat lower, due we think to the presence of a little vicilin which might be expected to be present, owing to the method of preparation. To facilitate comparison the other five analyses are arranged in the following table together with those of 5 and 8 described in our former paper.

LEGUMELIN.

	5	8	41	42	43	44	45	Aver.
Carbon	53.33	53.54	53.24	53.36	53.03	53.43	53.23	53.31
Hydrogen	6.98	6.99	6.99	6.98	7.10	7.01	6.91	6.99
Nitrogen	16.14	16.69	16.15	16.24	16.30	16.50	16.08	16.30
Sulphur	1.00	1.01	1.09	1.01	1.01	1.16	1.11	1.06
Oxygen	22.55	21.77	22.53	22.41	22.56	21.90	22.75	22.34
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

5 and 8, coagulated by alcohol.

41, coagulated by heat at 67°.

42, coagulated by heat at 95°.

43, coagulated by dialysis in water.

44, precipitated by acetic acid from ten per cent. salt solution.

45, precipitated by acetic acid from saturated salt solution.

The properties and reactions of legumelin will be discussed later after describing its occurrence in other seeds.

Returning to the first extract, the filtrate from 42 contained nothing coagulable on boiling. It was therefore dialyzed in alcohol and so concentrated to small volume. A quantity of proteid was thus precipitated which was filtered out and washed with absolute alcohol. The substance thus obtained, weighing 10.5 grams after drying over sulphuric acid, was dissolved in 100 cc. of water, with which it gave a clear solution, and sodium chloride was added to saturation, which caused a slight precipitate. This was filtered out, but owing to its small amount was not further examined. The filtered solution was then treated with salt-saturated acetic acid as long as a precipitate was produced, which required a relatively large amount of acid. The resulting gummy precipitate was washed with saturated salt solution, dissolved in water, the solution neutralized with a little sodium carbonate and dialyzed till free from chlorides, when the dialyzer was transferred to alcohol and the solution concentrated to small volume. The substance thus precipitated was filtered out, extracted thoroughly with absolute alcohol and dried over sulphuric acid giving 4.06 grams of preparation 46, having, when dried at 110°, the composition here given.

PROTEOSE, 46.

Carbon	50.24
Hydrogen	6.96
Nitrogen	17.35
Sulphur	1.25
Oxygen	24.40
	100.00
Ash	0.92

The solution, filtered from 46, which had been thrown down by the addition of acetic acid to the solution saturated with salt, was neutralized with sodium carbonate, of which a large quantity was required, showing that only a small proportion of the acid had been precipitated with the proteose. This neutralized solution was dialyzed in water until free from chlorides and then con-

centrated by dialysis in alcohol. The substance thus precipitated was filtered out, washed with absolute alcohol, dried and found to weigh 1.76 grams. This, dried at 110°, gave the following results when analyzed :

PROTEOSE, 47.

Carbon	49.66
Hydrogen	6.78
Nitrogen	16.57
Sulphur	1.40
Oxygen	25.59
	100.00
Ash	3.20

Preparations 46 and 47 give precipitates with copper sulphate which yield the usual rose-red biuret reaction on adding potash. With nitric acid they give no precipitates but yield yellow solutions on warming. If the solution is first saturated with salt, 46 gives a heavy precipitate with nitric acid which largely dissolves on heating and reprecipitates on cooling, while 47 under like conditions gives only a turbidity diminished by adding an excess of acid.

We have then in the pea the following proteids :

Legumin, a globulin not coagulated by heating its solutions.

LEGUMIN.

Carbon	51.74[1]
Hydrogen	6.90
Nitrogen	18.04
Sulphur	0.42
Oxygen	22.90
	100.00

Vicilin, a globulin soluble in a more dilute brine than legumin, coagulated on heating its solutions to 95°–100° and having the following composition :

VICILIN.

Carbon	52.36
Hydrogen	7.03
Nitrogen	17.40
Sulphur	0.18
Oxygen	23 03
	100.00

[1] This figure for carbon is about four-tenths per cent. lower than that given in our former paper, a difference which we attribute to the fact that this preparation was made from a neutralized solution and that vicilin had been separated from it completely.

Legumelin, a proteid partially precipitated by dialysis, generally in a coagulated state, the greater part however remaining in solution even after prolonged dialysis. Whether it should be called a globulin or an albumin is perhaps questionable, but its relations seem to be closer to the albumins than to the globulins. Its composition as shown by the average of the analyses of five preparations obtained by three different methods, is:

LEGUMELIN.

Carbon	53.31
Hydrogen	6.99
Nitrogen	16.29
Sulphur	1.06
Oxygen	22.35
	100.00

A *Protoproteose* precipitated by acetic acid from its solution saturated with salt:

PROTOPROTEOSE.

Carbon	50.24
Hydrogen	6.76
Nitrogen	17.35
Sulphur	1.25
Oxygen	24.40
	100.00

A *deuteroproteose* not precipitated by acetic acid from the salt saturated solution:

DEUTEROPROTEOSE.

Carbon	49.66
Hydrogen	6.78
Nitrogen	16.57
Sulphur	1.40
Oxygen	25.59
	100.00

The combined amount of legumin and vicilin which we have obtained by dialyzing extracts of the pea was about ten per cent., as the following experiments show.

Of the finely ground pea meal, 200 grams were treated with one liter of water, the mixture poured on a coarse sieve and the

lumps broken up and washed through with another liter of water. After standing over night, in a cool place, protected with thymol, the suspended matters settled leaving a somewhat turbid solution, of which 1500 cc. were siphoned off and filtered on a pulp filter. The first 300 cc. which passed through the filter were rejected, as they were mixed with the water contained in the pulp. The next liter of clear solution was dialyzed until all that could be thus precipitated had separated in the form of minute spheroids. In this way there was obtained a quantity of proteid which weighed 4.35 grams when washed with alcohol and dried over sulphuric acid. The extract from which this was separated was one-half the total and therefore corresponded quite nearly to that yielded by 100 grams of meal, thus showing that 4.35 per cent. thereof was extracted by distilled water and precipitated by dialysis.

The meal residue (from which, as described, three-fourths of the extract had been siphoned), together with the still adhering one-fourth of the extract, was treated with ten per cent. salt solution until the volume was 2000 cc. After settling, the solution was siphoned off, filtered perfectly clear, and dialyzed until all the globulin was precipitated. In this way 9.83 grams of globulin were obtained. As the meal residue before this extraction contained one-fourth of the water extract there should have been present 2.18 grams of proteid soluble in water and precipitable by dialysis. As three-fourths of the total salt extract yielded the above 9.83 grams there should be deducted therefrom three-fourths of 2.18 grams or 1.64 grams, making 8.19 grams of globulin obtained from three-fourths of 200 grams of meal or 5.46 per cent. which added to 4.35 per cent. makes 9.81 per cent. total globulin.

To another lot of 100 grams of meal 500 cc. of water were added, containing just enough baryta to produce an extract reacting neutral to litmus and to this 500 cc. of ten per cent. salt solution were added. From 750 cc. of this extract filtered clear, 7.33 grams of globulin separated on dialysis, equal to 9.77 per cent. of the meal. In another experiment, carried out in essentially the same manner, 10.0 per cent. of globulin was obtained.

By treating the residual meal with alkali but very little more proteid was dissolved. On digesting pea meal with ten times its

weight of one-tenth per cent. soda solution, a gelatinous mass resulted from which no clear extract could be obtained. If however a baryta solution of equal molecular strength was used the extract was readily filtered. From such an extract, filtered perfectly clear, there was obtained by adding acetic acid in slight excess 10.56 per cent. of proteid and by dialyzing the filtrate therefrom 3.16 per cent. more. Whether this 13.72 per cent. of proteid was substantially all legumin and vicilin we have no means of knowing. In the first of these experiments it will be noticed that 4.35 per cent. of globulin was obtained by dialyzing the aqueous extract. As the water used in this extraction weighed ten times as much as the meal the saline solution resulting from the mineral constituents of the seed would be so exceedingly dilute as to make it seem doubtful that so much globulin could be thereby dissolved. The reactions of this extract were therefore studied with a view to determine if possible the cause of the solution of the proteid.

When the extract was cautiously poured into distilled water a faint cloud formed at the point of contact of the two liquids which wholly disappeared on shaking. Under similar conditions a globulin solution commonly gives a permanent turbidity. Sodium chloride carefully and very gradually added, beginning with a minute quantity, gave no trace of a precipitate until the solution was saturated with the salt, when a very little proteid separated. A little acetic acid gave a precipitate completely dissolving on adding sodium chloride. If however a somewhat greater quantity of acid was added the precipitate did not wholly dissolve. By cautiously adding very dilute sodium carbonate solution even up to strong alkaline reaction no trace of a precipitate was observed.

Baryta solution when added in considerable quantity gave a little precipitate, probably due to phosphates. Calcium chloride gave a slight precipitate soluble in an excess of calcium or sodium chlorides. Calcium sulphate gave no precipitate.

From this it will be seen that solution of the globulin is apparently not due to the presence of acids, for neutralization gives not even a turbidity, nor to the presence of neutral salts since dilution does not give a permanent precipitate.

An extraction, with water containing just enough baryta to

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.[1]

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[2]. Liebig[3] 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
	100.00

Ritthausen[5] 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
	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

[1] Reprinted from advance sheets of the Report of the Connecticut Agricultural Experiment Station for 1897. Communicated by the authors.

[2] Gehlen's *J. der Chem.*, 6, 543.

[3] *Ann. Chem. Pharm.*, 39, 138.

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

[5] Die Eiweisskörper, etc., Bonn, 1872.