Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.	Milligrams copper.	Milligrams starch or dextrin.
320..	154.1	349..	169.0	378..	184.3	407..	199.6	436..	215.2
321..	154.7	350..	169.6	379..	184.7	408..	200.1	437..	215.7
322..	155.1	351..	170.1	380..	185.3	409..	200.7	438..	216.3
323..	155.7	352..	170.6	381..	185.8	410..	201.2	439..	216.8
324..	156.1	353..	171.1	382..	186.3	411..	201.8	440..	217.4
325..	156.7	354..	171.6	383..	186.9	412..	202.3	441..	217.9
326..	157.2	355..	172.1	384.	187.4	413..	202.8	442..	218.5
327..	157.7	356..	172.7	385..	188.0	414..	203.3	443..	219.1
328..	158.2	357..	173.2	386..	188.4	415..	203.9	444..	219.6
329..	158.7	358..	173.8	387..	189.0	416..	204.4	445..	220.2
330..	159.2	359..	174.2	388..	189.5	417..	205.0	446..	220.6
331..	159.8	360..	174.8	389..	190.0	418..	205.4	447..	221.2
332..	160.3	361..	175.4	390..	190.5	419..	206.0	448..	221.7
333..	160.8	362..	175.8	391..	191.1	420..	206.5	449..	222.3
334..	161.3	363..	176.4	392..	191.6	421..	207.1	450..	222.8
335..	161.8	364..	176.9	393..	192.1	422..	207.6	451..	223.4
336..	162.4	365..	177.5	394..	192.6	423..	208.2	452..	223.9
337..	162.8	366..	177.9	395..	193.2	424..	208.7	453..	224.4
338..	163.4	367..	178.5	396..	193.8	425..	209.3	454..	225.0
339..	163.9	368..	179.0	397..	194.3	426..	209.8	455..	225.6
340..	164.4	369..	179.5	398..	194.8	427..	210.3	456..	226.0
341..	165.0	370..	180.0	399..	195.3	428..	210.9	457..	226.6
342..	165.4	371..	180.6	400..	195.9	429..	211.4	458..	227.1
343..	166.0	372..	181.1	401..	196.4	430..	212.0	459..	227.7
344..	166.4	373..	181.6	402..	197.0	431..	212.5	460..	228.3
345..	167.0	374..	182.1	403..	197.4	432..	213.1	461..	228.8
346..	167.5	375..	182.7	404..	197.9	433..	213.6	462..	229.4
347..	168.0	376..	183.2	405..	198.5	434..	214.2	463..	230.0
348..	168.5	377..	183.7	406..	199.1	435..	214.7		

THE PROTEIDS OF LUPIN SEEDS.[1]

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

Received April 12, 1897.

THE lupin is a leguminous plant little known in this country except as a garden ornament. The yellow lupin (*Lupinus luteus*) and the blue lupin (*Lupinus angustifolius*), both native to Mediterranean regions, have long been cultivated in Europe because of their ability to grow luxuriantly on sandy or gravelly soils, and by their help large areas of poor, "worn out" land have been reclaimed and made agriculturally profitable, as these plants furnish abundant fodder and by the decay of their deeply penetrating roots, and especially when plowed under

[1] From the Report of the Connecticut Agricultural Experiment Station for 1896.

green, they rapidly impregnate the soil with humus and render it productive for other crops.

Ritthausen[1] first studied and described under the name *conglutin*, the characteristic proteid of the lupin seed. He found that the yellow and the blue lupin both yielded conglutin scarcely distinguishable in properties and only differing in composition as respects sulphur, of which his preparations from the yellow lupin contained one per cent. and those from the blue lupin but one-half per cent. Ritthausen also analyzed preparations which, from their composition, he concluded to be legumin. He stated that both legumin and conglutin are extracted from lupin seeds by salt solutions as well as by dilute alkali, and that the two proteids can be separated from each other by dissolving in alkali, then precipitating with an acid and finally treating with salt solution. On neutralizing these alkali solutions conglutin retains, while legumin loses its solubility in saline solutions.

By extracting with five per cent. sodium chloride brine Ritthausen obtained two preparations from yellow lupin having the composition given under 1 and 2; by dissolving 2 in potash water and reprecipitating, 3 was prepared; the blue lupin by extracting with salt solution yielded 4.

CONGLUTIN, RITTHAUSEN.

	Yellow Lupin.			Blue Lupin.
	1	2	3	4
Carbon	50.40	50.58	50.26	50.39
Hydrogen	7.00	7.06	6.89	6.94
Nitrogen	18.34	18.04	18.28	18.22
Sulphur }	24.26	24.32	{ 1.01	0.49
Oxygen }			{ 23.56	23.96
	100.00	100.00	100.00	100.00

The proteid rendered insoluble in salt solution by previous precipitation from an alkaline liquid, by acetic acid, he found to have the following composition, these figures being the average of five quite closely agreeing analyses:

LEGUMIN, RITTHAUSEN.

Carbon	51.36
Hydrogen	6.97
Nitrogen	17.50
Sulphur	0.59
Oxygen	23.58
	100.00

[1] *J. prakt. Chem.*, 103, 78; *Ibid*, New Series, 24, 222, and 26, 422, and *Die Eiweisskörper*, etc., *Bonn*, 1872.

Palladin[1] has recently described the properties of "vitellin" contained in seeds of the yellow lupin. In many details the results of his work do not agree with ours, but as he admits that the "vitellin" which he examined was always somewhat impure, it is unnecessary to review his statements here.

YELLOW LUPIN.

We find that seeds of the yellow lupin contain a small quantity of proteid that is soluble in pure water, a large quantity soluble in salt solutions, a small amount soluble in potash water, and a little nitrogenous matter, presumably proteid, which cannot be extracted by these solvents.

To determine the proportions of these proteids, very finely ground yellow lupin meal was first completely extracted with warm alcohol of 0.868 sp. gr. in order to remove, as far as possible, the amides and alkaloids which occur in considerable quantities in this seed. The air-dried meal before exhaustion with alcohol contained 8.16 per cent. of nitrogen. Alcohol removed eleven per cent. of the meal and gave a residue containing 8.49 per cent. of nitrogen, showing that six-tenths per cent. of nitrogen calculated on the original meal had been removed. The original meal accordingly contained 7.56 per cent. of nitrogen in the form of compounds insoluble in alcohol. As the seed reacted strongly acid towards litmus it is possible that a considerable quantity of alkaloids was not removed by the alcohol, but it was not desirable to add any alkali in this extraction for fear of affecting the proteids.

One thousand grams of the alcohol-exhausted and thoroughly air-dried meal were treated with successive quantities of ten per cent. sodium chloride solution until saturation of the filtered extract with ammonium sulphate showed that no more proteid was removed. The united extracts were filtered clear and saturated with ammonium sulphate; the precipitated proteids were dissolved in brine and the solution filtered and dialyzed for several days. The abundant precipitate thus produced was filtered out, washed by decantation with water and with alcohol and dried over sulphuric acid. The substance thus obtained weighed 279 grams.

The solution filtered from this globulin was saturated with

[1] *Ztschr. fur. Biol.*, 31, 191.

ammonium sulphate, the precipitate produced was dissolved in water and the solution filtered and dialyzed until no more globulin separated. By this second dialysis 15.2 grams of globulin were secured. The solution filtered from this second portion of globulin was concentrated by dialyzing in alcohol and the proteid completely thrown down by adding absolute alcohol. The precipitate which resulted was then dehydrated with absolute alcohol and dried over sulphuric acid. Only 4.20 grams of substance were thus obtained, indicating the presence of very little proteid soluble in water.

The residue of meal was washed with water, which took up no proteid, and exhausted by successive applications of two-tenths per cent. potash water. The alkaline extracts were filtered clear and treated with dilute acetic acid. No precipitate resulted until a decided excess of acid had been added. The proteid thus separated was washed with water and with alcohol, dried over sulphuric acid and weighed 60.53 grams.

The meal residue was next washed with water and with alcohol, dehydrated with absolute alcohol and thoroughly air-dried. It weighed 192.0 grams, showing the lupin seed to contain over eighty per cent. of substance soluble in salt solution and very dilute alkali, an amount not approached by any other seed which we have examined.

The nitrogen was determined in the various products and the results are summarized in the following table:

AMOUNT OF PROTEID EXTRACTED FROM YELLOW LUPIN BY VARIOUS SOLVENTS.

		Grams.
Extracted residue contained 1.51 per cent. N. or		2.90
Proteid extracted by alkali,	60.53 grams, contained 16.43 per cent. N. or	9.95
Proteid extracted by salt solution	279.0 grams, contained 17.86 per cent. N. or	49.83
	15.2 grams, contained 18.09 per cent. N. or	2.75
Proteid extracted by water,	4.2 grams, contained 16.55 per cent. N. or	0.69
Total proteid, 358.93 grams.		
Total nitrogen accounted for		66.12
1,000 grams of meal contained 8.49 per cent. N. or		84.90

We thus have 77.88 per cent. of the nitrogen accounted for.

We will next consider in order:

I. Proteids soluble in pure water.

II. Proteids insoluble in water, but soluble in sodium chloride solution.

III. Proteids insoluble in water and in salt solution, but soluble in dilute potash water.

I. *Proteids Soluble in Pure Water.*

As just indicated, water-soluble proteids occur in yellow lupin seeds in very small amount. Much the largest quantity obtained in any of the numerous extractions made was that already described, which formed 0.42 per cent. of the alcohol-extracted meal or 0.37 per cent. of the original meal. On treating this substance with water, a very considerable part was found to be coagulated. Since proteoses are not supposed to be rendered insoluble by prolonged treatment with alcohol, this insoluble matter was probably coagulated albumin or globulin, or possibly a mixture of both. The aqueous solution filtered from this insoluble proteid yielded a very small flocculent coagulum at 59°. The solution filtered after heating to 65°, became turbid at 67°, and flocks in minute amount appeared at 69°, which steadily increased until at 85° a very considerable coagulum had formed.

In another extraction by means of water, prolonged dialysis caused the separation of a small quantity of globulin which, when dissolved in salt solution, yielded a flocculent coagulum at 59° and but traces above 65°.

The solution from which most of this globulin had been removed by dialysis, gave a slight coagulum at 59°, and after heating to 65° the filtrate became turbid at 66°, and at 84° again yielded a small flocculent coagulum.

It is probable, therefore, that the substance coagulating at 59° is a globulin soluble in extremely dilute salt solutions which it is impossible to separate completely by dialysis and that the proteid coagulating at the higher temperature is an albumin.

Owing to the exceedingly small quantity of these proteids nothing further was learned respecting them.

The solution from which the coagulated proteid had been removed by heating gave a strong rose-red reaction with the

biuret test, but no precipitate in the cold with nitric acid even after adding salt.

The yellow lupin seed accordingly contains a very small amount of albumin and a small quantity of proteose.

Neumeister[1] states that he found peptone in lupin seeds in large amount.

In order to test the seeds of the yellow lupin for peptones, 1,000 grams of freshly ground meal were treated with three liters of distilled water, and after agitating therewith for an hour the extract was strained through fine bolting-cloth and the residue pressed out in a powerful screw press. Two liters of extract rich in dissolved substances was obtained, which was immediately saturated with ammonium sulphate and filtered. Lest peptones might be formed during the operation of extraction, the process up to this point was carried forward as rapidly as possible so that not over three hours elapsed before the solution, saturated with ammonium sulphate, was filtered. In order to be sure that the solution had been thoroughly saturated, a quantity of crystals of this salt were suspended in it over night, but no more proteid separated. Neumeister has stated that saturating seed extracts while hot and when made alternately acid and alkaline in reaction, is unnecessary. We, however, heated the solution to boiling, added ammonium sulphate as long as it dissolved, concentrated somewhat and allowed to cool. Much ammonium sulphate separated, but no noticeable quantity of proteose. After filtering off the separated sulphate, the solution was heated to boiling and concentrated until sulphate began again to crystallize out. Ammonia was next added to distinct alkaline reaction, and after heating a short time the solution was again cooled, filtered from deposited sulphate, and the filtrate concentrated until more sulphate separated. Acetic acid was added to acid reaction, the heating continued for a time, and the whole again cooled. After filtering out the separated crystals the solution measured 350 cc. The solution was then nearly neutralized with ammonia, leaving its reaction slightly acid, and after adding an equal volume of water a freshly prepared solution of tannic acid was gradually added so long as a precipitate was produced in a small portion of the filtered liquid. A bulky reddish precipitate

[1] *Ztschr. für. Biol., n. f.*, 12, 461.

formed which, after standing twenty-four hours, was removed from the filter and treated with a slight excess of hot concentrated solution of baryta. After standing a short time the warm solution was filtered, and as it was very strongly alkaline from free ammonia, one-half was neutralized with sulphuric acid, thereby removing the excess of baryta. Neutral lead acetate was then added and the solution filtered. The most carefully applied biuret test did not show the least trace of peptone in this solution. The remainder was then evaporated nearly to dryness and about two cc. of syrup obtained which, if it had all been peptone, would hardly be considered a large amount. No peptone reaction whatever could be obtained with this syrup.

The other half of the solution was treated exactly as Neumeister directed—that is, neutral lead acetate was added without neutralizing the ammonia, and, after filtering, the biuret reaction was applied, but with no indication of peptone.

Since writing the above, S. Frankfurt[1] has stated that no peptone is present in seeds of the lupin, and attributes Neumeister's results to his long treatment of the seeds with water. In a letter to Frankfurt, Neumeister stated that after swelling the seeds in water they were rubbed up and digested with water for twenty-four hours, and that the vessels containing the extracting seeds were exposed to the direct action of sunlight in summer. It is thus evident that the peptone found by Neumeister was formed during the extraction and was not an original constituent of the seed.

II. *Proteids Soluble in Saline Solutions.*

As just mentioned, a very small quantity of a globulin soluble in extremely dilute salt solutions, yielding a flocculent coagulum when its solution in ten per cent. sodium chloride is heated to 59°, was found in extracts of the yellow lupin seed. As but little of this proteid is present in this seed, no attempt was made to do more than note its presence. Owing to its ready solubility in very dilute salt solutions, this proteid dissolves when lupin meal is treated with water. Besides this little if any other globulin substance is extracted from lupin seeds by the dilute saline solution which results when the meal is treated with water. Large

[1] *Landw. Ver.-Stat.*, 47, 454.

quantities of globulin are, however, obtained by extraction with stronger salt solutions.

One hundred grams of meal yielded directly with salt solution an extract which was filtered clear, saturated with ammonium sulphate, the precipitate produced filtered out, dissolved in brine, and the solution filtered clear and dialyzed for eighteen hours. The globulin thus precipitated was filtered out, washed with water, alcohol, and ether, dried over sulphuric acid, and found to weigh twelve grams. The filtrate by further dialysis similarly yielded four and seven-tenths grams of globulin. These preparations, 1 and 2, were analyzed with the results given below. The residue, remaining after exhausting 100 grams of meal with water, on treating with salt solution gave an extract which, when filtered clear and dialyzed four days, yielded 20.36 grams of globulin, preparation 3, having the composition given in the following table. A large quantity of the globulin was prepared by extracting one kilogram of finely ground lupin meal with six liters of brine, filtering the resulting solution, saturating with ammonium sulphate, filtering out the precipitate produced, dissolving this in dilute salt solution, again precipitating by saturating with ammonium sulphate, redissolving the precipitate, filtering the solution so obtained and dialyzing for forty hours. The very large precipitate which separated was washed thoroughly by decantation with water, dilute alcohol, absolute alcohol and ether and then dried over sulphuric acid. In this way 112 grams of preparation 4 were secured having the composition given in the subjoined table. The filtrate from 4, after three days further dialysis, gave a second precipitate of globulin which was decidedly more viscid than 4. This by the usual treatment yielded 30 grams of preparation 5 with the composition given below.

Another portion of meal weighing one kilogram was several times extracted with water and the residue treated with successive applications of ten per cent. salt solution. The extract was filtered clear and dialyzed for forty hours. The globulin so precipitated was thoroughly washed with water by decantation, then with dilute alcohol and then with absolute alcohol until no more color was removed, and finally with ether and then dried over sulphuric acid; 120 grams of preparation 6 were thus

obtained, giving on analysis the figures stated below. The solution from which 6 had separated was saturated with ammonium sulphate, the precipitate dissolved in brine and the solution returned to the dialyzer. After some days the small precipitate which had separated was filtered out, washed and dried in the usual manner and gave five grams of preparation 7 having the composition given below.

In order to avoid as far as possible any contamination of the proteid with nitrogenous or other substances soluble in alcohol, a quantity of very fine ground lupin meal was exhausted in Squibb's percolator with a large quantity of strong alcohol, the process being continued until only a trace of solid matter remained after evaporating a considerable quantity of the alcoholic extract.

Two kilograms of this meal were then extracted as thoroughly as possible with brine, the solution filtered clear and saturated with ammonium sulphate. The proteids thus precipitated were dissolved in brine, the solution filtered perfectly clear and the globulin thrown down by dialyzing three days was filtered off, washed and dried in the manner already described. There was thus obtained 506 grams of globulin, preparation 8.

The solution filtered from 8 by longer dialysis yielded forty-five grams of preparation 9. These preparations had the composition given below.

LUPIN GLOBULIN.

	1.	2.	3.	4.	5.	6.	7.	8.	9.
Carbon	50.62	50.68	50.63	50.49	50.29	50.41	49.81	50.20	49.47
Hydrogen ..	6.94	6.95	7.00	6.77	6.89	6.85	6.79	6.75	6.77
Nitrogen ...	17.45	17.89	18.05	17.90	17.88	18.01	17.79	17.86	18.07
Sulphur	0.77	0.80	0.88	0.88	1.25	0.87	1.48	0.98	1.49
Oxygen.....	24.22	23.68	23.44	23.96	23.69	23.86	24.13	24.21	24.20
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Ash.....	0.51	0.27	0.74	0.26	0.30	0.99	0.83	0.38	0.23

These figures agree quite closely except those for 5, 7, and 9 in which carbon is less and sulphur decidedly more than in the others. The fact that these three preparations separated on prolonged dialysis of the solutions which had yielded 4, 6, and 8 indicates the presence of two globulins of different composition.

It was therefore necessary to submit this substance to very thorough fractional precipitation in order to determine definitely its true composition. Accordingly, 100 grams of preparation 6

were dissolved in 800 cc. of five per cent. salt solution, filtered from a very small quantity of insoluble matter, and the perfectly clear solution was mixed with 800 cc. of water and cooled to 0°. A very large proportion of the dissolved proteid separated as a solid, coherent mass on the bottom of the beaker, from which the clear solution was completely decanted. This precipitate was marked *A*, the solution *B*. The precipitate *A* was dissolved in 100 cc. of ten per cent. brine, yielding readily a perfectly clear solution. This was mixed with 100 cc. of water at 20° but no precipitate resulted. Water was then added gradually until an abundant precipitate formed and the total volume equalled 500 cc. After settling, the clear solution was poured off from the precipitate and the latter washed thoroughly with water and alcohol and dried over sulphuric acid. In this way 46 grams of preparation 10 were obtained, having the following composition :

LUPIN GLOBULIN, 10.

Carbon	50.49
Hydrogen	6.77
Nitrogen	17.89
Sulphur	0.66
Oxygen	24.19
	100.00
Ash	0.51

The solution decanted from 10 was mixed with 600 cc. more water, which gave another precipitate that, after washing and drying, weighed ten and a half grams and had the composition given below.

LUPIN GLOBULIN, 11.

Carbon	50.18
Hydrogen	6.94
Nitrogen	17.93
Sulphur	0.82
Oxygen	24.13
	100.00
Ash	0.77

The solution *B* decanted from the first precipitate *A*, as described above, was diluted with an equal volume of water, cooled to 0°, and allowed to deposit the resulting precipitate. After washing and drying, preparation 12, weighing eight

grams, was obtained, which gave the following results on analysis :

LUPIN GLOBULIN, 12.

Carbon	50.08
Hydrogen	6.82
Nitrogen	18.26
Sulphur	1.30
Oxygen	23.54
	100.00
Ash	0.74

The solution from which 12 separated was saturated with ammonium sulphate, the proteid thereby precipitated was dissolved in water and the clear solution dialyzed. A small precipitate resulted, which when washed and dried, weighed one and four-tenths grams and had the following composition :

LUPIN GLOBULIN, 13.

Carbon	49.54
Hydrogen	6.91
Nitrogen	18.24
Sulphur } Oxygen }	25.31
	100.00
Ash	0.49

These fractional precipitations show a regular decrease in their content of carbon and an increase in both nitrogen and sulphur, that of the latter being especially marked. It is to be noted that the total weight of the foregoing fractions formed only 65.9 per cent. of the proteid taken, suggesting that the globulin was undergoing a change while in solution ; but as no especial care was exercised to obtain all the proteid from the solutions, this process was repeated, not only with a view to settling this point, but to obtain larger quantities of the extreme fractions for further examination.

One hundred grams of preparation 8 were dissolved in 800 cc. of five per cent. brine and the solution, after filtering perfectly clear, was mixed with 800 cc. of water, and cooled to 10°. The abundant precipitate which resulted was allowed to settle, and the solution decanted. The precipitate was marked *C*, the solution *D*. Precipitate *C* was next dissolved in 100 cc. of ten per cent. brine and 300 cc. of water at 20° added to the resulting

solution. After standing some time the clear solution was decanted from the large deposit of proteid and the latter washed and dried. Thus were obtained fifty grams of preparation 14, which analysis showed to have the following composition :

LUPIN GLOBULIN, 14.

Carbon	50.71
Hydrogen	7.00
Nitrogen	17.86
Sulphur	0.67
Oxygen	23.76
	100.00
Ash	0.39

The filtrate from 14 was mixed with 400 cc. of the first washings of 14 and cooled to 7°–8°. On standing, a part of the proteid deposited and the clear solution was then decanted. The precipitate, preparation 15, after washing and drying weighed 8.46 grams, and according to analysis contained :

LUPIN GLOBULIN, 15.

Carbon	50.14
Hydrogen	6.94
Nitrogen	17.83
Sulphur	0.86
Oxygen	24.23
	100.00
Ash	0.81

The solution from which 15 had separated was dialyzed, but only a very small quantity of globulin could be obtained, preparation 16, which weighed seven-tenths gram and without correction for ash contained 18.22 per cent. of nitrogen. Solution *D* decanted from precipitate *C*, as already described, was cooled to 3°, that is, 7° lower than before. This caused a further quantity of globulin to separate, which gave preparation 17, weighing 21.14 grams.

LUPIN GLOBULIN, 17.

Carbon	50.13
Hydrogen	6.88
Nitrogen	17.72
Sulphur	0.80
Oxygen	24.47
	100.00
Ash	0.59

The solution from which 17 separated was dialyzed free from chlorides and 13.7 grams of preparation 18 obtained, having the following composition :

LUPIN GLOBULIN, 18.

Carbon	50.04
Hydrogen	6.79
Nitrogen	18.43
Sulphur	1.48
Oxygen	23.26
	100.00
Ash	0.25

The filtrate from 18 contained but a very little proteid precipitable with ammonium sulphate. The total weight of these fractions was ninety-four grams; the six grams unaccounted for may be fairly attributed to mechanical loss and therefore a change of proteid to non-proteid substances is improbable.

These fractions, like those of the preceding series, show a decrease in carbon and an increase in nitrogen and sulphur as we pass from the least to the most soluble.

Another series of separations was next made by fractional solution.

One hundred grams of preparation 8 were dissolved in 800 cc. of five per cent. brine diluted with 800 cc. of water at 20° and the solution cooled to 5°. The clear solution was then decanted from the separated proteid and dialyzed till free from chlorine. The globulin thus separated after washing and drying weighed 24.03 grams and had the following composition :

LUPIN GLOBULIN, 19.

Carbon	50.11
Hydrogen	6.84
Nitrogen	18.46
Sulphur	1.28
Oxygen	23.31
	100.00
Ash	0.17

The proteid deposited by cooling, as just described, was dissolved in 700 cc. of five per cent. brine and cooled at 5°. The clear solution was decanted and dialyzed, yielding 18.60 grams of preparation 20, which contained :

LUPIN GLOBULIN, 20.

Carbon	50.27
Hydrogen	6.78
Nitrogen	18.43
Sulphur	1.15
Oxygen	23.37
	100.00
Ash	0.10

The substance precipitating on cooling, as just described, was dissolved in 600 cc. of five per cent. salt solution, mixed with an equal volume of water, and cooled to 2°. The clear solution was dialyzed and five and six-tenths grams of preparation 21 were obtained, which gave the following results on analysis:

LUPIN GLOBULIN, 21.

Carbon	50.03
Hydrogen	6.86
Nitrogen	18.47
Sulphur	1.49
Oxygen	23.15
	100.00
Ash	0.22

The precipitate that separated on cooling the solution from which 21 was obtained, was dissolved in 500 cc. of five per cent. brine, diluted with an equal volume of water, and cooled to 7°. The solution was decanted from the precipitate and dialyzed. Thus was obtained 4.31 grams of preparation 22, which contained:

LUPIN GLOBULIN, 22.

Carbon	50.44
Hydrogen	6.92
Nitrogen	18.23
Sulphur	1.14
Oxygen	23.27
	100.00
Ash	0.11

The substance deposited by cooling, as last described, was dissolved in 300 cc. of five per cent. salt solution, filtered clear and dialyzed till free from chlorides. The deposited globulin, after washing and drying, weighed 33.88 grams and contained:

LUPIN GLOBULIN, 23.

Carbon	51.13
Hydrogen	6.86
Nitrogen	18.03
Sulphur	0.49
Oxygen	23.49
	100.00
Ash	0.52

Like the preceding, this series of fractional separations shows a decrease in carbon and an increase in nitrogen and sulphur with increased solubility. The total weight of the fractions obtained was 86.42 grams, the loss being no greater than was to be expected.

Thirty-five grams of preparation 10 and the same quantity of preparation 14, representing the least soluble portions obtained in the two first series of fractions, were next dissolved in 600 cc. of five per cent. brine, diluted with an equal volume of water, and cooled to 6°. The solution was decanted from the precipitate which resulted, filtered clear, and dialyzed. The globulin thus precipitated after washing and drying weighed 12.43 grams and contained:

LUPIN GLOBULIN, 24.

Carbon	50.10
Hydrogen	6.94
Nitrogen	18.12
Sulphur	0.94
Oxygen	23.90
	100.00
Ash	0.23

The precipitate produced by cooling the solution, as just described, was dissolved in 500 cc. of salt solution, diluted with 500 cc. of water and cooled to 6°. The deposited proteid, after the usual treatment, weighed 7.80 grams and had the following composition:

LUPIN GLOBULIN, 25.

Carbon	50.32
Hydrogen	6.90
Nitrogen	18.06
Sulphur	0.81
Oxygen	23.91
	100.00
Ash	0.27

The substance separated by cooling, as just described, was dissolved in one liter of two and one-half per cent. sodium chloride solution and cooled to 7°. The clear liquid, decanted from the thus precipitated proteid, was dialyzed and yielded 5.51 grams of globulin, giving, on analysis, the following results:

LUPIN GLOBULIN, 26.

Carbon	50.80
Hydrogen	6.91
Nitrogen	18.01
Sulphur	0.64
Oxygen	23.64
	100.00
Ash	0.23

The proteid separated by cooling the solution from which 26 had been obtained was dissolved in half a liter of five per cent. salt solution, diluted with an equal volume of water, and cooled to 14°. The clear solution was decanted and dialyzed and gave four and nine-tenths grams of preparation 27, which analysis showed to have the composition here given:

LUPIN GLOBULIN, 27.

Carbon	50.90
Hydrogen	6.85
Nitrogen	17.88
Sulphur	0.55
Oxygen	23.82
	100.00
Ash	0.27

The substance deposited at 14°, as noted above, was dissolved in half a liter of five per cent. brine, diluted to one liter, and allowed to settle at the temperature of the room (about 22°). The liquid was then decanted, dialyzed, and further treated in the usual manner. There was thus obtained 5.45 grams of preparation 28, which contained:

LUPIN GLOBULIN, 28.

Carbon	50.93
Hydrogen	6.94
Nitrogen	17.83
Sulphur	0.48
Oxygen	23.82
	100.00
Ash	0.23

The substance separated by diluting the solution from which 28 resulted, was washed with water and alcohol, dried, and found to weigh 20.6 grams. Its composition was :

LUPIN GLOBULIN, 29.

Carbon	50.80
Hydrogen	6.83
Nitrogen	17.88
Sulphur	0.46
Oxygen	24.03
	100.00
Ash	0.70

This, like the other series of fractional separations, shows an increase in carbon and decrease in sulphur and nitrogen content accompanying a decrease in solubility. If the final fractions, which have been most thoroughly purified, are arranged as in the table below, it will be seen that a nearly constant composition has been reached so that the average of these analyses may be taken as closely representing the composition of the least soluble and most abundant globulin of the yellow lupin.

YELLOW LUPIN GLOBULIN, CONGLUTIN.

	25.	26.	27.	28.	29.	Average.
Carbon	51.13	50.80	50.90	50.93	50.80	50.91
Hydrogen	6.86	6.91	6.85	6.94	6.83	6.88
Nitrogen	18.03	18.01	17.88	17.83	17.88	17.93
Sulphur	0.49	0.64	0.55	0.48	0.46	0.52
Oxygen	23.49	23.64	23.82	23.82	24.03	23.76
	100.00	100.00	100.00	100.00	100.00	100.00

This is the globulin discovered by Ritthausen, and described by him under the name conglutin.

A careful examination of preparation 29 showed this globulin to have the following reactions :

In very dilute acids and alkalies it is completely soluble, yielding perfectly clear solutions of a light yellow color.

Dissolved in very dilute acetic acid, the proteid is precipitated by neutralizing the solution with sodium carbonate. On adding sodium chloride brine to the solution containing the precipitate in suspension, the latter is completely dissolved.

In ten per cent. sodium chloride brine it dissolves readily, giving a very slight turbid solution of a pale yellow color, which

turns litmus paper red, lacmoid paper blue, and has no effect on tropæoline when evaporated to dryness with it.

A solution containing ten per cent. of the globulin dissolved in ten per cent. sodium chloride brine behaved as follows :

Dilution with twice its volume of water produced a considerable precipitate.

Addition of one drop of acetic acid, sp. gr. 1.035, or one drop of hydrochloric acid (one part concentrated acid and three parts of water) to five cc. of this solution gave a heavy precipitate.

Addition of mercuric chloride[1] dissolved in ten per cent. brine gave no precipitate.

Tannin as well as picric acid gave a heavy precipitate.

Diluted with an equal volume of ten per cent. brine the solution, containing five per cent. of globulin, reacted as follows :

No precipitate was produced by saturation with sodium chloride, magnesium sulphate or sodium sulphate at 20°, but at 34° saturation with the last named salt precipitated all but a minute trace of the globulin.

When the solution in ten per cent. sodium chloride brine, containing five per cent. of proteid, was heated gradually in a water-bath, no change appeared even after heating some time at 100°. After more prolonged heating a thick transparent skin formed on the surface. On cooling and standing several hours, the solution set to a solid jelly and became somewhat turbid.

Addition of nitric acid to the solution in brine gave a precipitate not soluble in an excess of this acid. On heating, the usual xanthoproteic reaction occurred, which was preceded by the development of a slight pink color quickly changing to yellow, doubtless due to a trace of coloring matter still adhering to the proteid. When the globulin in the dry state was treated with very dilute nitric acid a clear solution resulted which gave a heavy precipitate on adding an excess of acid, that behaved on heating as just described. The usual proteid reactions were obtained with Millon's, Adamkiewics', and the biuret tests.

In order to determine the composition of the more soluble fractions, the greater parts of 18, 19, and 20 were united, giving

[1] Palladin (Ztschr. für Biol., 31, 195) states that ten per cent. sodium chloride solutions are not precipitated by mercuric chloride, but that diluted solutions give precipitates soluble in salt solution. The precipitate which he thus obtained was of course caused by the water added with the mercuric chloride. If he had dissolved the mercury salt in brine, no precipitate would have resulted.

forty grams of substance which was dissolved in 400 cc. of five per cent. brine, the solution was filtered perfectly clear, the filter washed with 100 cc. of the same salt solution, and the filtrate and washings were mixed with an equal volume of water. After cooling to 8°, and standing some time, a part of the globulin separated. From this the solution was decanted. The proteid was deposited as a viscid transparent fluid, which became opaque on treating with distilled water and finally solid. It was dehydrated with absolute alcohol and dried over sulphuric acid, giving 19.2 grams of preparation 30, having the following composition :

LUPIN GLOBULIN, 30.

Carbon	49.64
Hydrogen	6.87
Nitrogen	18.21
Sulphur	1.20
Oxygen	24.08
	100.00
Ash	0.32

The solution decanted from preparation 30 was mixed with 500 cc. of water and cooled to 7°. The resulting precipitate, when treated in the same way as 30 had been, gave 9.25 grams of preparation 31, containing :

LUPIN GLOBULIN, 31.

Carbon	49.62
Hydrogen	6.72
Nitrogen	18.22
Sulphur	1.36
Oxygen	24.08
	100.00
Ash	0.43

The solution from which 31 had separated was dialyzed free from chlorides, and yielded seven and four-tenths grams of preparation 32 :

LUPIN GLOBULIN, 32.

Carbon	49.59
Hydrogen	6.75
Nitrogen	18.43
Sulphur	1.62
Oxygen	23.61
	100.00
Ash	0.17

These three fractions have very nearly the same composition, which is in close agreement with that of the other most soluble fractions already described, as may be seen from the following table :

LUPIN GLOBULIN.

	13.	18.	9.	30.	31.	32.	Average.
Carbon	49.54	49.63	49.47	49.64	49.62	49.59	49.58
Hydrogen...	6.91	6.78	6.77	6.87	6.72	6.73	6.80
Nitrogen....	18.24	18.43	18.08	18.21	18.22	18.43	18.27
Sulphur .. }	25.31	1.48	1.49	1.20	1.36	1.56	1.42
Oxygen... }		23.68	24.20	24.08	24.08	23.67	23.93
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

A series of tests of preparation 30, conducted at the same time and under identical conditions with those described for preparation 29, revealed the following differences. In ten per cent. sodium chloride brine, 30 dissolved to a perfectly clear solution more colored than that yielded by 29.

Preparation 30, dissolved in brine to a solution containing ten per cent. both of salt and proteid, was not even rendered turbid by dilution with two volumes of water, but with three volumes a slight precipitate was given. A similar solution of 29, with two volumes of water, gave a considerable precipitate, and with three volumes a very heavy precipitate.

Five cc. of the ten per cent. solution of 30 required eight drops of acetic acid, sp. gr. 1.035, to produce a slight precipitate, while one drop under the same conditions gave a heavy precipitate in a similar solution of 29.

With one drop of hydrochloric acid (one part concentrated hydrochloric acid and three parts water) only a turbidity was produced in the case of 30, while under like conditions 29 yielded a heavy precipitate.

Saturation with sodium sulphate of the solution containing five per cent. of 30 gave only a partial precipitation, 29 being wholly precipitated under like conditions.

When this five per cent. solution in ten per cent. brine was heated in a water-bath a turbidity formed at 94°, increasing as the temperature was raised, becoming dense at 99°, and after long heating a gelatinous flocculent precipitate separated which was unchanged on cooling and standing ; when heated with

nitric acid the pink color which preceded the yellow of the xanthoproteic reaction was more pronounced than that yielded by 29.

In all other respects no difference could be detected between the reactions of 30 and 29. The most noticeable property in which both differ from nearly all other vegetable globulins is that neither yields insoluble products (the so-called "albuminates") when separated from solution. The only evidence of such a tendency noticed in the very large number of preparations made, was in some cases shown by the presence of a slight turbidity when precipitates were redissolved.

Both give solutions which react strongly acid with litmus. Titrated with standard ammonia, two grams of each of these globulins required the addition of ten milligrams of ammonia to cause an alkaline reaction with litmus.

Although the analyses of the final fractions of the more soluble globulin agree closely and their properties and reactions are quite alike, it seems to us doubtful if they represent a definite chemical species. The close physical and chemical resemblance between the least soluble substance and the most soluble, suggests that they are closely related. The distinct difference in some properties observed between the extreme products of fractional precipitation make it probable that a combination of some sort has taken place between the conglutin and other constituents of the seed.

As the lupin seed is unusually rich in soluble constituents, it would not be surprising if the proteid on precipitation carried down with it more or less of these substances which could be with difficulty separated afterwards. From the globulin of the blue lupin by fractional precipitation readily soluble products were obtained which however were wholly different in composition and quite distinct in reaction from the more soluble fractions of the yellow lupin globulin. The soluble fractions are possibly conglutin combined either chemically or mechanically with other constituents of the seed, which in the two varieties of lupin are apparently present in different proportions.

III. *Proteid Insoluble in Water and Saline Solution but Soluble in Dilute Alkalies.*

As previously described on page 457 after exhausting one kilogram of yellow lupin meal with ten per cent. sodium chloride

solution and washing the residue with water, by continued extraction with two-tenths per cent. potash water a solution was obtained which after filtering clear and adding acetic acid to distinct acid reaction yielded a precipitate which after washing with water and alcohol and drying over sulphuric acid weighed 60.53 grams. This was redissolved in two-tenths per cent. potash water, the solution again filtered clear and carbon dioxide passed through it. The proteid partly separated, but could not be filtered, so a little ammonium chloride was added to convert the potassium carbonate into chloride, but even after passing carbon dioxide through the solution for some time and allowing it to stand over night in an ice box, only a partial separation resulted. Acetic acid was then added in slight excess and the precipitate filtered off and washed thoroughly with water and with alcohol. Dried at 110° this preparation, 33, gave the following results on analysis :

LUPIN PROTEID, 33.

Carbon	51.40
Hydrogen	6.79
Nitrogen	16.43
Sulphur	1.03
Oxygen	24.35
	100.00
Ash	1.57

BLUE LUPIN.

One kilogram of fine ground meal of the seeds of the blue lupin was extracted with a large quantity of distilled water applied in successive portions and the residual meal thrown on fine bolting cloth after each application. The extract thus obtained was allowed to stand over night. The partly clarified liquid was siphoned off, saturated with ammonium sulphate, the precipitate dissolved in brine, and the solution filtered clear and dialyzed for forty-eight hours. A precipitate resulted which was filtered off, washed with water and with alcohol, and dried over sulphuric acid, giving preparation 34, weighing 8.46 grams. The filtrate was dialyzed for ten days longer in a stream of water, but no more proteid separated. The solution was then concentrated by dialysis in alcohol and absolute alcohol added until all the proteid contained in it was precipitated. The sub-

stance thus obtained weighed, when dry, only 1.42 grams and was not examined further than to find that it was nearly all insoluble in water, having been coagulated by the prolonged treatment with alcohol. This insolubility of the greater part of this substance shows that very little proteose is present.

Like the yellow lupin, this seed contains but little proteid matter soluble in water, and that is mostly globulin dissolved by aid of the salts extracted from the seed.

The meal residue which had been exhausted with water was treated with ten per cent. sodium chloride solution and the extract, after filtering perfectly clear, was dialyzed for forty hours. The globulin, which separated in a coherent mass on the bottom of the dialyzer, was washed thoroughly by decantation with water and with alcohol and dried over sulphuric acid. This gave 115.0 grams of preparation 35.

Another preparation of this globulin was made by treating one kilogram of the meal directly with ten per cent. brine, filtering the extract perfectly clear, and dialyzing for forty hours. The globulin thus precipitated, after washing and drying, formed preparation 36 and weighed 112 grams. These three preparations were dried at 110° and analyzed with the following results:

BLUE LUPIN GLOBULIN, CONGLUTIN.

	34.	35.	36.	Yellow lupin.
Carbon	50.58	50.82	50.85	50.91
Hydrogen	6.58	6.87	6.78	6.88
Nitrogen	17.82	18.04	18.04	17.93
Sulphur	0.72	0.40	0.50	0.52
Oxygen	24.30	23.87	23.83	23.76
	100.00	100.00	100.00	100.00
Ash	0.64	1.22	1.11	

The agreement between 35 and 36 and the purified conglutin of the yellow lupin is very close indeed, but in order to be sure that this was not accidental these two preparations were subjected to the following treatment:

One hundred grams of 35 were dissolved in one-half liter of five per cent. sodium chloride brine and one-half liter of water added to the solution. A large rapidly settling precipitate resulted, which formed a semi-fluid mass on the bottom of the beaker, from which the very nearly clear solution *A* was poured

after a short time. The precipitate *B* was dissolved in 280 cc. of six and a half per cent. brine and the resulting solution, which measured 380 cc., was diluted with an equal volume of water and cooled to 5°. The solution was then decanted from the precipitate, which was washed and dried as usual and found to weigh 39.2 grams, preparation 37. The solution decanted from 37 was dialyzed until free from salt, whereby a precipitate resulted which, when washed and dried, weighed four and two-tenths grams and formed preparation 38. The solution *A*, decanted from precipitate *B*, as above described, was cooled to 5° and the solution *C* decanted from the precipitate *D*. This precipitate was again dissolved in 300 cc. of five per cent. brine, 300 cc. of water added, and the whole cooled to 5°. The precipitate thus produced, when dried, weighed 19.5 grams, preparation 39. The solution from which 39 had separated was dialyzed and yielded preparation 40, weighing five and four-tenths grams.

The solution *C* decanted from precipitate *D* was dialyzed free from chlorides and thereby thirteen grams of preparation 41 were separated. These preparations were dried at 110° and analyzed with the following results. In the table they are arranged according to their solubility in dilute salt solutions.

	37.	39.	38.	40.	41.	
Carbon	51.25	51.04	50.82	50.98	50.79	
Hydrogen	6.96	6.75	6.66	6.83	6.79	
Nitrogen	18.11	18.15	17.69	17.66	17.64	
Sulphur	0.32	0.24	0.49	0.38	0.39	
Oxygen	23.36	23.82	24.34	24.15	24.39	
	100.00	100.00	100.00	100.00	100.00	
Ash	0.95	0.71	0.61	0.52	0.75	
Amount	39.2	19.5	4.2	5.4	13.0	grams

From these figures it would seem that preparation 35 contained some impurities, which accumulated in the three most soluble fractions in which the nitrogen is a little lower and the sulphur slightly higher than in the less soluble fractions. Preparation 36 was next fractionally precipitated in the following manner:

One hundred grams were dissolved in 700 cc. of five per cent. sodium chloride brine, filtered perfectly clear, and the solution diluted with an equal volume of distilled water. The proteid

separated as a viscid liquid from which the solution *E* was decanted. The precipitate *F* was dissolved in 100 cc. of five per cent. brine and the resulting solution, which measured 175 cc., was cooled in a freezing mixture to —4°, and then allowed to stand until it had warmed to 20°. The precipitate thus produced formed a perfectly transparent syrupy liquid which measured 61 cc. The solution from which this had separated was decanted and the fluid precipitate was washed by stirring up with water, whereby it was rendered opaque and pasty. On washing with fresh quantities of water the substance became denser and granular. It was finally washed with alcohol and dried over sulphuric acid, giving twenty-five grams of preparation 42.

The washings of 42 were cooled to 0° and four-tenths gram of preparation 43 obtained. The solution from which this separated contained very little proteid, which was not saved. The solution *E*, decanted from precipitate *F*, was cooled to 8°, and the solution *G*, was decanted from the proteid *H*, thus separated.

This precipitate was treated with seventy-seven cc. of five per cent. sodium chloride brine giving a solution measuring 126 cc., which was cooled to —10°, and then allowed to stand until warmed to 20°, when forty-nine cc. of a clear viscid liquid separated. This was washed with water in the same manner as 42 had been, and then with alcohol, dried over sulphuric acid, and 18.5 grams of preparation 44 obtained. From the washings of 44 by cooling to 0°, 2.62 grams of preparation 45 were separated. The solution *G*, decanted from precipitate *H*, was cooled in a freezing mixture until partly frozen, when it was allowed to melt and deposit the separated proteid. The latter, *J*, after decanting the solution *I*, was dissolved in fifty cc. of five per cent. sodium chloride brine and the seventy-five cc. of solution which resulted was cooled to —2°, but only a turbidity resulted. The solution was therefore diluted with an equal volume of water, again cooled to —2°, and slowly warmed to 20°. The proteid separated as a viscid liquid measuring twenty-four cc., and when washed with water and alcohol gave ten grams of preparation 46. From the washings of 46, by cooling to 0°, there separated 0.56 gram of preparation 47. The solution *I*,

decanted from precipitate *J*, was diluted with an equal volume of water, and cooled to 0°. The substance which separated was washed with water and alcohol and when dried weighed 9.15 grams, and formed preparation 48. The solution from which 48 had separated contained too little proteid to save.

Dried at 110° these preparations gave the following results on analysis, which are arranged in the table in the order of their solubility.

	42.	44.	43.	45.	46.	47.	48.	
Carbon....	51.09	51.14	....	50.86	50.94	....	50.65	
Hydrogen.	6.83	6.89	....	6.89	6.89	....	6.84	
Nitrogen..	18.08	18.10	17.82+	17.95	17.79	17.77	17.56	
Sulphur ..	0.38	0.33	....	0.46	0.27	....	0.44	
Oxygen...	23.62	23.54	....	23.84	24.11	....	24.51	
	100.00	100.00		100.00	100.00		100.00	
Ash	0.59	0.47	?	0.69	0.51	0.54	0.86	
Amount ..	25.00	18.50	0.40	2.62	10.00	0.56	9.15	gms.

If the analyses of preparations 37, 39, 42, and 44, which constitute the greater part of the proteid substance of 35 and 36, are compared it will be seen that they are in very close agreement, and it is fair to presume that they represent very nearly the true composition of this, the principal proteid of the blue lupin. If these analyses are also compared with those of conglutin from the yellow lupin, it will be evident that the two varieties of lupin contain one and the same globulin, especially since a rigid comparison of the reactions of purified preparations from the two seeds failed to reveal the slightest difference. The following table will facilitate a comparison of the above-mentioned figures:

	CONGLUTIN.					
	Blue lupin.					Yellow lupin.
	37.	39.	42.	44.	Average.	Average.
Carbon	51.25	51.04	51.09	51.14	51.13	50.91
Hydrogen....	6.96	6.75	6.83	6.89	6.86	6.88
Nitrogen.....	18.11	18.15	18.08	18.10	18.11	17.93
Sulphur	0.32	0.24	0.38	0.33	0.32	0.52
Oxygen.....	23.36	23.82	23.62	23.54	23.58	23.76
	100.00	100.00	100.00	100.00	100.00	100.00

If now we compare the analyses of the more soluble fractions, as shown in the following table, they will be seen to be quite

similar to each other but decidedly different from the more soluble globulin of the yellow lupin:

	38.	40.	41.	46.	47.	48.	Average.	Soluble globulin yellow lupin.
Carbon ...	50.82	50.98	50.79	50.94	....	50.65	50.84	49.58
Hydrogen.	6.66	6.83	6.79	6.89	....	6.84	6.80	6.80
Nitrogen .	17.69	17.66	17.64	17.79	17.77	17.56	17.69	18.27
Sulphur ..	0.49	0.38	0.39	0.27	....	0.44	0.39	1.42
Oxygen ...	24.34	24.15	24.39	24.11	....	24.51	24.28	23.93
	100.00	100.00	100.00	100.00		100.00	100.00	100.00

A comparison of the reactions of 41 with those of 37 showed that much less difference existed between the extreme fractions from the blue lupin than between those from the yellow. A ten per cent. solution of 37 in ten per cent. brine gave a considerable precipitate when diluted with twice its volume of water, while three times its volume were required to produce a slight precipitate in similar solutions of 41. Solutions of both were precipitated with equal quantities of acid, 41 not needing the large excess of acid to cause precipitation which the soluble product from the yellow lupin required.

A solution of five per cent. of 37, in ten per cent. brine, even after prolonged heating at 99°–100°, appeared wholly unaffected until the solution was subsequently cooled, when it solidified. A similar solution of 41 began to yield a flocculent coagulum at 75°, which at 80° was voluminous. After heating in a boiling water-bath for some time nearly all the proteid was coagulated. As to the relations of these two substances, what was said on page 474, in our opinion, applies equally in this case.

CONCLUSION.

Both yellow and blue lupin seeds contain very little proteid matter soluble in water. The total quantity of proteid soluble in pure water obtained from the yellow lupin amounted to only 0.37 per cent. Of this a part consists of proteose. Whether the remainder is albumin, or a globulin soluble in extremely dilute salt solutions, which therefore could not be completely separated by dialysis, was not determined. Peptone is not contained in the freshly ground seed but is formed in small quantity after prolonged contact with water.

The greater part of the proteid matter contained in these seeds is soluble in saline solutions, the yellow lupin yielding 26.2 per cent. This is the body known as conglutin, but as heretofore described and as usually obtained it is contaminated with other substances present in the seed. Preparations from the blue lupin are usually much purer than those from the yellow, for the latter contain a considerable quantity of some sulphur-containing substance from which conglutin can be separated by fractional precipitation out of dilute salt solutions. This explains why Ritthausen's conglutin from the yellow lupin contained twice as much sulphur as that from the blue lupin.

When purified no difference in properties and reactions can be detected between preparations from the two seeds.

The composition of conglutin, as obtained by us, is shown by the following figures :

CONGLUTIN.

	Yellow lupin.	Blue lupin.
Carbon	50.91	51.13
Hydrogen	6.88	6.86
Nitrogen	17.93	18.11
Sulphur	0.52	0.32
Oxygen	23.76	23.58
	100.00	100.00

Conglutin is readily soluble in sodium chloride solutions containing upwards of five per cent. of the salt. By sufficient dilution it is precipitated, a syrupy liquid separating which is rendered opaque and solid by treatment with water. Dissolved in salt solution, it is apparently unaffected by prolonged heating in a boiling water-bath, but solutions thus heated on standing and cooling form a solid opalescent jelly which becomes clear and fluid on again heating. Unlike other globulins conglutin does not yield insoluble (coagulated) products by washing with alcohol or drying.

After exhausting lupin meal with salt solution, a small quantity of proteid can be extracted by two-tenths per cent. potash water, from which it is precipitated by adding acetic acid in slight excess but not by making the solution neutral to litmus. Only one preparation of this substance was made, which gave the following results on analysis :

Carbon	51.40
Hydrogen	6.79
Nitrogen	16.43
Sulphur	1.03
Oxygen	24.35
	100.00

Owing to the insolubility of this substance in any but alkaline fluids and the difficulty of making preparations of known purity, nothing further was learned respecting it.

EFFECT OF MINUTE QUANTITIES OF ACID ON THE SOLUBILITY OF GLOBULIN IN SALT SOLUTIONS.[1]

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

Received April 12, 1897.

IN a paper on crystallized vegetable proteids by one of us[2] it is shown that the principal globulin of the seed of the castor bean is partly insoluble in a saturated solution of sodium chloride, and partly soluble therein, and that these two parts are alike in composition and but slightly different in reactions. Having found a proteid of similar composition and properties in the sunflower seed, we have again turned our attention to the globulin of the castor bean, with the hope that we might discover the cause of this partial precipitation by saturating its solutions with salt.

A considerable quantity of this globulin was prepared by extracting castor pomace with three per cent. brine at 60° and allowing the filtered extract to cool to the temperature of the room. The proteid thus separated was washed with water and alcohol, and dried over sulphuric acid. It formed a slightly colored dense powder consisting of a mixture of spheroids and octahedral crystals.

Seventy-five grams of this preparation were treated with 750 cc. of ten per cent. salt solution, and after agitating for some time, filtered from a large insoluble residue. This latter was washed thoroughly with ten per cent. brine and the filtrate and washings were united. In this way the substance was separated into two parts, one soluble and one insoluble, in cold salt solution. This solution was then saturated with sodium chloride

[1] From the Report of the Connecticut Agricultural Experiment Station for 1896.

[2] Osborne: *Am. Chem. J.*, 14, 671.