PROTEOSE, 22.

Carbon	50.85
Hydrogen	6.75
Nitrogen	16.65
Sulphur Oxygen	25.75
	100.00

THE PROTEIDS OF THE PEA, LENTIL, HORSE BEAN, AND VETCH.[1]

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As shown in our papers on the proteids of the pea, lentil, horse bean, and vetch, these seeds contain legumin, legumelin, and proteose, and the three first-named also contain vicilin. A careful comparison of the reactions and properties of these proteids, as now obtained by us, reveals no difference between preparations of the same substance from different seeds.

LEGUMIN.

Legumin forms the chief proteid constituent obtainable from the vetch, pea, lentil, and horse bean. In the first-named seed about ten per cent. of the meal was found to consist of legumin; in the three other seeds this proteid is associated with vicilin, from which we have no method for its quantitative separation. From the pea about ten per cent., from the lentil thirteen per cent., and from the horse bean about seventeen per cent. of these mixed proteids were obtained. The lentil contains the least proportion of legumin, which seems to form about two-thirds of the mixed proteids, while the horse bean contains the greatest, as in this seed vicilin is present in relatively small amount.

Legumin is a globulin, for it dissolves readily in saline solutions and is precipitated therefrom either by dialysis, dilution, or cooling. By dialysis or by cooling it separates in the form of spheroids which, after settling from the solution, unite to form a plastic mass. By diluting its concentrated solutions the legumin separates as a viscid, translucent fluid. This fluid, when

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treated with water, becomes opaque and solid, so that the legumin can be converted into a coarse meal by rubbing with a glass rod under water. Conglutin from lupin seeds and amandin from almonds behave similarly, as do gliadin of wheat and rye and hordein of barley, when precipitated from alcoholic solutions by dilution with water.

Solutions containing more than two per cent. of sodium chloride dissolve legumin abundantly; those containing less salt have a solvent power rapidly decreasing with the diminishing salt content so that a one per cent. salt solution dissolves very little. Saturation with sodium chloride or magnesium sulphate does not precipitate legumin from its solution in brine, but saturation with sodium sulphate at 34° precipitates it almost completely.

In pure water legumin is entirely insoluble, but if the solution from which the legumin is precipitated contains acid, this may combine with the legumin and the resulting preparation, like other acid globulins, will then dissolve in pure water.

If seeds containing legumin are extracted with water, more or less of the legumin is dissolved; from the pea about four per cent., from the vetch two and a half per cent., from the lentil ten per cent., and from the horse bean sixteen per cent. The legumin thus dissolved is largely precipitated by dialysis in water, by the addition of acids and by lime salts and very slightly by great dilution with water. These aqueous extracts react strongly acid with litmus, and alkaline with lacmoid, a behavior doubtless due to acid potassium phosphates together with organic acids or acid salts, for acids combined with proteids do not react with lacmoid, although they readily turn blue litmus red. Solutions which we have made, of legumin from various seeds as well as of edestin from hemp-seed, by dissolving the proteid in monohydrogen potassium phosphate, have shown us all the reactions of these aqueous extracts excepting one presented by the horse bean, the unneutralized water extracts of which are precipitated with *pure* sodium chloride. This reaction we cannot explain, for these extracts give with dilute acids precipitates soluble in salt solutions and no precipitates on neutralization, reactions which seem to exclude the presence of acid globulin.

Solutions of legumin or of edestin in monohydrogen potassium phosphate behave more like solutions in dilute alkali carbonates than like solutions in neutral salts, for they give precipitates with dilute acids which are soluble in more acid or in salt solutions and are not precipitated by dilution unless very little phosphate is present. If phosphoric acid is added to the potassium phosphate solution, the solvent power of the phosphate is diminished and the facility with which the proteid is precipitated by dilution is increased; but if only little acid is added, yet enough to give a decided reaction with litmus, considerable quantities of the globulins are still dissolved and the reactions of solutions so made closely resemble those of the aqueous extracts of these seeds. As a result of numerous comparisons of the reactions of solutions so prepared with those of the aqueous extracts of leguminous seeds, we believe that the extraction of legumin by water from these seeds is due to the presence of acid potassium salts of phosphoric and organic acids, and that in consequence of the varying proportions of these substances in the different kinds of seeds, different amounts of legumin are thus extracted from them. As the proportion of phosphoric acid to potash in these leguminous seeds is much smaller than in most of the other seeds which we have examined, the character of the salts present may fairly be supposed to differ, and consequently the solubility of the proteids would also differ when the seeds are extracted with water. Thus in lupin seeds the amount of phosphoric acid, one and four-tenths per cent., exceeds that of the potash, one and one-tenth per cent., and from them water extracts but little proteid. Liebig and Ritthausen attributed the solubility of legumin to *basic* phosphates, and the latter undertook extensive experiments to show the presence of an excess of potash in the aqueous extracts, but such a supposition appears to overlook the fact that the aqueous extracts of these seeds are decidedly acid towards litmus.

Dissolved in dilute sodium chloride solution, legumin is precipitated by a little acetic acid, the precipitate being soluble in an excess of sodium chloride. The solubility of precipitates so produced depends on the relative proportions of salt and acid.

Legumin extracted without neutralizing the natural acid of the seed and precipitated by dialysis, either directly or after precipitation with ammonium sulphate, is usually converted to a large extent into insoluble " albuminate."

This " albuminate" differs from the similar insoluble products obtained from most other globulins, for when treated with salt solution it becomes gelatinous, cannot be filtered, and, on washing with water, shrinks, becomes opaque, and finally granular, so that it can be very readily washed on a filter. This substance appears to become hydrated by salt solution and dehydrated by pure water.

If the acid of the seed is previously neutralized, the globulin extracted by salt solution yields very little if any insoluble " albuminate," which indicates that the latter is a product of the action of the acid of the seed. This fact is in harmony with experiments described in a former paper of ours on the action of minute quantities of acid on globulins.[1] In very dilute acids and alkalies in absence of salts, legumin dissolves readily and abundantly, from which solutions, if at once neutralized, it is precipitated in a form soluble in sodium chloride solution. By this treatment no evidence of change has been detected. Solutions made with hydrochloric acid are precipitated by a small excess of acid, but those made with acetic acid are not precipitated by any excess of acid.

Solutions of legumin in ten per cent. sodium chloride brine are not rendered turbid by long heating in a boiling water-bath.

Dissolved in ten per cent. sodium chloride brine, legumin is precipitated by a very little hydrochloric acid, but a relatively considerable amount of acetic acid is required to produce a precipitate in such solutions.

Sodium chloride solutions of legumin give large precipitates with tannic acid as well as with picric acid, those formed by the latter dissolving in an excess of salt solution if too much picric acid had not been previously added. With mercuric chloride no precipitate is produced.

With nitric acid, Millon's and Adamkiewicz's tests, proteid reactions are obtained. With the biuret test a violet color is given which on standing becomes a deep rose-red like that given by peptones.

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[1] Report of the Connecticut Agricultural Experiment Station for 1896, p. 369. This Journal, 19, 482.

Legumin has been supposed by some investigators to contain phosphorus and therefore to belong with the nucleo-proteids. A careful testing of thoroughly purified samples, by fusing with caustic soda and nitrate, and treating the solution of the fusion acidified with nitric acid with ammonium molybdate, showed that in some of the preparations only just detectable traces of phosphorus were present, while other preparations contained none whatever.

Although we have examined large numbers of our preparations of the different plant proteids for phosphorus, we have as yet found none which, in carefully purified samples, contained more than a few hundredths of a per cent., a quantity so small that it is reasonable to consider it as a constituent of the ever-present ash.

The composition of legumin is shown by the following figures, which are averages of a number of preparations from each of the different seeds:

LEGUMIN.

	Pea.	Lentil.	Horse bean.	Vetch.	Average.
Carbon	51.74	51.73	51.72	51.69	51.72
Hydrogen	6.90	6.89	7.01	6.99	6.95
Nitrogen	18.04	18.06	18.06	18.02	18.04
Sulphur	0.42	0.40	0.39	0.43	0.41
Oxygen	22.90	22.92	22.82	22.87	22.88
	100.00	100.00	100.00	100.00	100.00

VICILIN.

Vicilin is a globulin associated with legumin in the pea, lentil, and horse bean. But as we have no means of separating vicilin and legumin quantitatively, we can state nothing respecting the amount in which it occurs in these seeds further than that the lentil contains the most and the horse bean the least. In the lentil it probably forms about one-third of the mixed globulins. That vicilin is not a derivative of legumin, is almost conclusively proved by the fact that no vicilin can be obtained from the vetch.

The most remarkable characteristic of vicilin is its content of sulphur, less than that of any other known proteid. This element, it may be noticed, diminishes in quantity with repeated precipitation as though by this process sulphur were split off from the molecule. The total quantity, however, is so small that it might appear unsafe to draw conclusions from the differences observed, that is from 0.23 per cent. maximum to 0.08 per cent. minimum. Yet repeated determinations of this element have shown us that the differences were not analytical. It would seem possible by sufficiently repeated precipitation to obtain from this proteid preparations free from sulphur.

In salt solution vicilin is much more soluble than legumin, so that by repeated precipitation from diluted solutions the two globulins can be separated.

When solutions of vicilin in ten per cent. brine are heated in a water-bath they become turbid at 90° and at 95° flocks separate. When heated for some time at 100° this globulin is almost completely coagulated. In water, vicilin is insoluble. In one per cent. sodium chloride solution it dissolves considerably, while in slightly stronger solutions it is much more soluble, the solution of the globulin appearing to depend on the presence of enough salt to form a soluble compound.

In its other reactions it very closely resembles legumin.

The composition of vicilin we found to be as follows:

VICILIN.

	Pea.	Lentil.	Horse bean.	Average.
Carbon	52.36	52.13	52.38	52.29
Hydrogen	7.03	7.02	7.04	7.03
Nitrogen	17.40	17.38	17.52	17.43
Sulphur	0.18	0.17	0.15	0.17
Oxygen	23.03	23.30	22.91	23.08
	100.00	100.00	100.00	100.00

LEGUMELIN.

We have found legumelin in all the leguminous seeds which we have examined with the exception of the white bean (*Phaseolus vulgaris*) and the blue and yellow lupin.

It is difficult to decide whether this proteid should be considered an albumin or a globulin.

By prolonged dialysis of solutions containing considerable legumelin a very small part separates, usually in a form insoluble in salt solutions. In one case we obtained a precipitate by dialysis which was wholly soluble in brine, to a solution from which a relatively large coagulum separated on heating to 65°, thus indicating a globulin. We have found, however, that other proteids, notably edestin, undergo a change in solubility, whereby the proteid becomes gradually more and more difficult to dissolve. Thus edestin changes from a form insoluble in cold brine to one soluble in hot brine, also from one soluble in hot brine to one insoluble therein, but soluble in dilute sodium carbonate solution, and finally from a form insoluble in alkali carbonate to one soluble only in caustic alkali. It is not unreasonable to expect that a proteid which might properly be considered an albumin (*i. e.*, soluble in pure water and coagulable on heating) should undergo a similar change whereby it becomes no longer soluble in pure water but dissolves in salt solutions and then further changes to a form no longer soluble in brine but dissolved by alkali carbonates.

Such a very large proportion of the legumelin resists precipitation by dialysis, that we are inclined to regard the small precipitates which we have obtained in this way as due to changes similar to those which produce so-called "albuminates" from globulins.

In a paper on the proteids of the cow pea we designated this proteid as a globulin, but in view of our subsequent, much more extensive experience we now consider legumelin to be more properly classed with the albumins.

The amount of legumelin which we have found in the seeds discussed in this paper, was in the pea two per cent., vetch one and one-half per cent., lentil and horse bean one and one-quarter per cent.

No definite coagulation point can be stated for legumelin, as the presence of salts or acids as well as the proportion of dissolved legumelin have a great effect on the temperature at which coagulation takes place. Coagulated legumelin is soluble in very dilute alkalies, as is shown by the following experiment tried with preparation 41 from the pea. About seven-tenths gram of this substance which had been nearly dried over sulphuric acid, but which still contained some alcohol, was suspended in fifty cc. of water and ten cc. of one-half per cent. caustic soda solution added. The legumelin dissolved at once, giving a glairy solution very similar in appearance to undiluted white of egg. After standing a few moments the viscidity disappeared and the solution became thin and clear. This solution was then almost neutralized by adding a nearly sufficient quantity of a dilute acetic acid which had been previously titrated against the soda solution. The liquid still remained clear and the legumelin was not precipitated until but a trace of the alkali remained unneutralized.

In dilute hydrochloric or acetic acid the coagulum is not soluble. By adding ten per cent. of sodium chloride to solutions containing legumelin, and then acetic acid, the acid compound of this proteid is precipitated, which dissolves in water to a solution that on neutralization gives a precipitate insoluble in water.

By saturating seed extracts, freed as far as possible from all globulin, with sodium chloride or magnesium sulphate only a very slight precipitate is obtained, showing that legumelin is thus slightly if at all precipitated.

Owing to the impossibility of separating legumelin from associated proteose, except in a coagulated state, we have learned but little respecting its reactions.

In the following table we give the average of analyses which we have made of coagulated legumelin from different seeds. These preparations have been obtained in a variety of ways, as can be seen by consulting our papers on the seeds named.

LEGUMELIN.

	Pea.	Lentil.	Horse bean.	Vetch.	Adzuki bean	Cow pea.	Soy bean.	Average.
Carbon ·····	53.31	53.22	53.03	53.31	53.97	53.25	53.06	53.31
Hydrogen ··	6.99	6.82	6.97	6.97	7.01	7.07	6.94	6.97
Nitrogen ···	16.30	16.27	16.22	16.24	16.31	16.36	16.14	16.26
Sulphur····	1.06	0.94	1.30	1.11	0.88	1.11	1.17	1.08
Oxygen ····	22.34	22.75	22.48	22.37	21.83	22.21	22.69	22.38
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Ritthausen recognized the presence of this proteid in the horse bean and pea, and gave analyses of coagula obtained by boiling extracts of these seeds. These analyses, however, do not agree well with each other and only in a general way with ours. On account of the solubility in alkali of the heat coagulum of this proteid, he did not consider it to be albumin.

PROTEOSE.

As the proteose of these seeds is present in small amount and is difficult to obtain pure, we have not as much information respecting it as is desirable. The pea appears to contain about one per cent., the horse bean about one-half per cent., and the lentil and vetch evidently less. It is probable that more or less of this proteose may be lost by diffusion, for 10.5 grams of what was doubtless nearly pure proteose from the pea, after solution and dialysis yielded only about six grams when reprecipitated. We have obtained a few of the reactions of proteose from the pea and horse bean, but no reactions of this proteid from the lentil and vetch.

By saturation with salt, solutions of the proteose of the pea and horse bean are not precipitated, but by subsequently adding salt saturated acetic acid, a large part of the pea proteose separates while all but a trace of that from the horse bean is thrown down. Nitric acid in the aqueous solutions of the pea proteose gives no precipitate unless the solution is previously saturated with salt, when a precipitate, soluble on warming and reappearing on cooling, is given by that part of the proteose precipitable by acetic acid from a salt saturated solution, while the part not thus precipitable gives only a turbidity. Both these parts of the pea proteose are precipitated by copper sulphate, and give a rose-red biuret reaction.

The composition of the preparations from these seeds was found as follows:

PROTEOSE.

	Pea.		Lentil.	Horse bean.		Vetch.
	46	47	68	89	98	22
Carbon	50.24	49.66	50.17	50.24	49.96	50.85
Hydrogen	6.76	6.78	6.77	6.66	6.76	6.75
Nitrogen	17.35	16.57	16.81	17.11	16.95	16.65
Sulphur	1.25	1.40	1.27	1.87	2.75	25.75
Oxygen	24.40	25.59	23.98	24.12	23.58	
	100.00	100.00	100.00	100.00	100.00	100.00

46, Precipitated by saturating with salt and adding acetic acid.

47, From the filtrate from 46.

68, Total proteose precipitated by alcohol.
89, Ditto.
98, Precipitated from salt saturated solution by acetic acid.
22, Total proteose precipitated by alcohol.

If the difficulty encountered in purifying these preparations of proteose and the different methods by which they have been obtained are considered, the agreement between them, except for the sulphur in those from the horse bean, makes it probable that these figures quite nearly represent the composition of this substance.

PROTEIDS OF THE SOY BEAN.[1] (*Glycine hispida.*)

By Thomas B. Osborne and George F. Campbell.

THE proteids of the soy bean, so far as we are informed, have never been the subject of special investigation. The seeds used by us were grown in Kansas and kindly supplied by Prof. C. C. Georgeson. Two varieties have been examined, one known as the yellow soy bean and the other, called in Japan, *kiyusuki diadzu.*

YELLOW SOY BEAN.

The seeds were first coarsely ground, then freed almost entirely from the outer coats by a current of air and finally ground to a fine flour.

From this meal, water dissolved a large quantity of proteid, yielding an extract exceedingly difficult to filter and impossible to make perfectly clear, for the solutions, even after repeatedly filtering through the densest filters, were always strongly opalescent.

The aqueous extract reacts acid with litmus, about 0.75 gram of caustic potash being required to produce a neutral reaction in an extract from 100 grams of meal. When thus neutralized no noticeable precipitate is produced, indicating the absence of acid proteid compounds. Dilute acids give abundant precipitates in the aqueous extract, as does the addition of lime salts, the precipitates produced by these reagents being readily soluble in sodium chloride solutions.

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