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.

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

Dialysis of the aqueous extract of 100 grams of meal precipitated 16.6 grams of globulin, which was largely soluble in brine. The solution filtered from this precipitate began to coagulate on heating to about 55°; after further heating to 80° the coagulum amounted to 1.53 grams. The reactions of the aqueous extract are like those similarly obtained from other leguminous seeds and indicate that the globulin is dissolved by means of the potassium phosphates contained in the seed.

To obtain the proteids soluble in sodium chloride solution, 2000 grams of meal were treated with ten liters of ten per cent. brine, the extract was strained through fine bolting-cloth and the residue squeezed in a screw press. The turbid, gummy extract, measuring about eight liters, was centrifugated until no more solid matter could be removed. The liquid was pumped through a thick bed of filter-paper pulp and yielded a reddish yellow solution, which appeared perfectly clear by transmitted light, but was opaque by reflected light. This extract was saturated with ammonium sulphate and a very bulky precipitate obtained which, after draining on filters over night, measured, when removed from the papers, over two liters. This precipitate was stirred up with a little water, strained through bolting-cloth to break up lumps, and dialyzed in running water over night. By this process the excess of ammonium sulphate, which had prevented solution, was so far removed that the proteid completely dissolved with the help of the remaining sulphate, in about three liters of water. The liquid was then filtered perfectly clear and again dialyzed for four days. The contents of the dialyzers were then allowed to deposit the large precipitate of globulin that had separated, and from this the solution A was siphoned off. The precipitate was collected on several filters, the contents of one were thoroughly washed in succession with water, alcohol, absolute alcohol, and ether, and dried over sulphuric acid. Preparation 1, weighing 25.11 grams, was thus obtained. The rest of the globulin was allowed to drain thoroughly on the filters and then dissolved in 750 cc. of five per cent. brine, yielding 1250 cc. of solution. To this was added 1000 cc. of water and the large precipitate produced, after standing about four hours, was filtered out and without washing was treated with ten per cent. brine. Nearly all dissolved, but a very slimy sub-

stance that remained suspended in the solution, made filtration practically impossible. The solution was therefore passed through the centrifugal separator, which removed a small quantity of viscid matter, and was then filtered perfectly clear through paper pulp and dialyzed for two days. The large precipitate which resulted was filtered out and washed with water. The washings at first were clear, but afterwards became turbid in consequence of the precipitation of dissolving proteid. The substance was then washed with dilute alcohol and finally with absolute alcohol and dried over sulphuric acid. This preparation, 2, weighed eighty grams. The aqueous washings of 2 were made clear by adding a little salt and then dialyzed. After two days the precipitate which separated was filtered out, washed with water and with alcohol, giving preparation 3, weighing 5.43 grams. The dialyzed solution from which 2 had separated contained very little proteid. The solution from which 2 had been originally separated by dilution was cooled over night at 7° and decanted from the precipitate that resulted, which formed a transparent layer of thick viscid fluid, as does legumin under similar conditions. This substance was treated with water, which rendered it opaque with separation of a white solid. After thoroughly washing with water the latter was dehydrated with absolute alcohol and dried over sulphuric acid, giving twenty grams of preparation 4. The solution decanted from 4, measuring 1650 cc., was treated with 2000 cc. of water, and after standing at rest for about five hours, was poured off from a semifluid deposit. This, after thoroughly washing with water and alcohol and drying, gave 18.76 grams of preparation 5.

The solution decanted from 5 was cooled to 5° for about eighteen hours, but as very little proteid separated, it was dialyzed for two days and then filtered from a small precipitate. The latter, washed with water and alcohol and dried over sulphuric acid, gave 5.37 grams of preparation 6.

A part of preparation 2 was mixed with five-tenths per cent. sodium carbonate solution, in which it was mostly soluble, and the resulting solution was neutralized with dilute acetic acid. Crystallized salt was then added, whereby the neutralization precipitate partially redissolved. The insoluble substance was filtered out and the clear filtrate dialyzed for forty-eight hours.

The proteid separated in spheroidal form and was filtered out, washed with water and with alcohol, making preparation 7.

A portion of preparation 4 was suspended in water and mostly dissolved by help of a little acetic acid. Dilute sodium carbonate solution was added until the proteid appeared to be completely precipitated, leaving the liquid distinctly acid to litmus. Crystallized salt was added, which gave a nearly complete solution of the precipitate. The solution was filtered and dialyzed for four days, when the precipitated globulin was filtered out, washed, and dried, giving preparation 8.

Another preparation was made from a neutralized extract, by treating 200 grams of meal with two liters of five per cent. salt solution to which had been added a quantity of baryta, previously determined to be sufficient to make the resulting extract neutral to litmus.

The mixture was strained through fine bolting-cloth and allowed to settle over night, but the suspended matter separated very imperfectly. The extract was then filtered on a pulp-filter with a pump, and 1500 cc. of a very nearly clear solution obtained, which was much less opalescent than any previously made from unneutralized extracts. This was saturated with ammonium sulphate and the resulting precipitate dissolved in water; the solution obtained was filtered perfectly clear and dialyzed for three days. The contents of the dialyzer were then filtered and the precipitate was washed with water. A portion of this substance was used to determine the reactions of this proteid and the remainder was washed with alcohol and dried, forming preparation 9.

All the preparations thus described were then dried to constant weight at 110° and analyzed with the following results:

GLYCININ: GLOBULIN OF YELLOW SOY BEAN.

	1	2	3	4	5
Carbon	52.04	51.73	51.84	51.94	51.90
Hydrogen	7.06	6.74	6.89	6.93	7.03
Nitrogen	17.14	17.72	17.46	17.52	17.44
Sulphur	0.77	0.76	0.79	0.73	0.66
Oxygen	22.99	23.05	23.02	22.88	22.97
	100.00	100.00	100.00	100.00	100.00
Ash	0.34	0.57	0.43	0.53	0.41

	6	7	8	9	Average
Carbon	51.75	51.65	51.71	52.12	51.85
Hydrogen	6.90	6.97	6.82	6.93	6.92
Nitrogen	17.03	17.63	17.57	17.53	17.45
Sulphur	0.67	0.66	0.61	0.79	0.72
Oxygen	23.65	23.09	23.29	22.63	23.06
	100.00	100.00	100.00	100.00	100.00
Ash	0.30	1.34	0.61	0.38	....

With the exception of the figures for nitrogen in the crude globulin, 1, and in the final small fraction, 6, these analyses are in close accord and give no evidence of any fractional separation. It is probable, therefore, that we have here a definite proteid distinct from legumin, containing one-half per cent. less nitrogen and three-tenths per cent. more sulphur than that substance. We propose for this globulin the name *Glycinin*. Its reactions, determined by an examination of preparation 9, are as follows:

In sodium chloride solutions it is readily soluble and is precipitated by dialysis, by dilution, and by cooling.

By dialysis or by cooling it separates in the form of spheroids which, according to circumstances, after settling from the solution either unite to a more or less coherent mass or else form a clear, transparent, viscid fluid, which, like legumin under similar conditions, is rendered white and solid by treating with water.

Solutions containing more than two per cent. of sodium chloride dissolve glycinin freely; the solvent power of those containing less salt diminishes more rapidly than the percentage of salt decreases.

Dissolved in sodium chloride solution, glycinin is not precipitated by saturating with magnesium sulphate or sodium chloride, but is completely precipitated by saturating with sodium sulphate at 34°.

In pure water, glycinin, when prepared from carefully neutralized extracts, is not soluble.

If soy bean meal is treated with water, upwards of sixteen per cent. of this globulin is dissolved, but in this case the solution is doubtless due to the potassium phosphates contained in the seed, as was pointed out in our paper on legumin.

Dissolved in ten per cent. sodium chloride solution, glycinin

is not coagulated even by prolonged heating in a boiling water-bath.

By dilute acetic acid a precipitate is formed insoluble in excess of salt solution. If, however, glycinin be dissolved in a solution containing but little salt, the precipitate produced by acetic acid is soluble in a slight excess of either acid or salt. The insoluble, so-called albuminate form of this globulin, like that of legumin, when treated with salt solution becomes gelatinous and it is impossible to filter solutions containing even a small quantity of it.

In absence of salts, glycinin is readily soluble in quite dilute acetic acid and is precipitated from such solution by sodium carbonate, even before the acid is wholly neutralized. When the acid is completely neutralized the precipitate obtained is entirely soluble in brine. Dissolved in ten per cent sodium chloride solution, glycinin is precipitated by tannic acid and by picric acid, the latter yielding a precipitate soluble in an excess of salt solution if too much picric acid be not present. With mercuric chloride no precipitate is produced. With Millon's Adamkiewicz's, the biuret and xanthoproteic test the usual proteid reactions result.

The solution A, page 420, which, as described, had been filtered from the globulin precipitated by dialyzing the solution of the ammonium sulphate precipitate of the total proteids of the extract, contained the remaining proteids extracted from the beans. This solution was saturated with ammonium sulphate, the precipitate so produced was filtered out and dissolved in a small quantity of water. After filtering clear, the solution was dialyzed for eight days. The globulin, which separated in well-formed spheroids, when washed and dried weighed twenty grams, preparation 10. The filtrate from 10 was saturated with ammonium sulphate, the precipitate dissolved in a little water, filtered clear, and dialyzed for five days; a small deposit appeared which when washed and dried weighed 2.71 grams, preparation 11. The solution filtered from 11 was returned to the dialyzer and, as nothing more separated after forty-eight hours, the dialyzer was transferred to alcohol. During the next forty-eight hours a considerable precipitate formed which when washed with alcohol and dried weighed 11.5 grams. Of this

substance seven and five-tenths grams were treated with water and the insoluble part was thoroughly washed with water and alcohol and dried. Preparation 12 was thus obtained, weighing four and seven-tenths grams. The filtrate, from the precipitation of this substance by dialysis in alcohol, was mixed with a large excess of alcohol and the precipitate caused thereby was filtered out, dehydrated with absolute alcohol, dried and found to weigh one and four-tenths grams, preparation 13. These preparations were dried at 110° and analyzed with the following results :

	10	11	12	13
Carbon	51.94	52.00	53.06	48.76
Hydrogen	6.88	6.83	6.94	6.28
Nitrogen	16.51	16.36	16.14	16.14
Sulphur	0.60	24.81	1.17	28.82
Oxygen	24.07		22.69	
	100.00	100.00	100.00	100.00
Ash	0.39	0.19	0.44	1.35

Of these preparations 10 and 11 have nearly the composition of phaseolin, but whether they are identical with that globulin could not be ascertained because they had become so largely coagulated by washing with alcohol and drying, that their reactions could not be satisfactorily determined.

A small quantity of globulin was obtained by further dialyzing the filtrate from preparation 9, page 422, which, when dissolved in ten per cent. salt solution, became turbid at about 95°, and after continued heating at 99°, gave a considerable coagulum. Hydrochloric or acetic acid added to its solution in ten per cent. brine gave no precipitate, and dilution with water gave a precipitate only when carried to a great extent. These reactions are characteristic of phaseolin, and taken in connection with the analyses of the similarly obtained preparations 10 and 11, make it probable that these seeds contain a small proportion of phaseolin.

Preparation 12 has the composition of legumin, which we have obtained in the same manner from a number of other leguminous seeds.

Preparation 13 is proteose.

SOY BEAN. (*Kiyusuki Diadzu.*)

Fine flour made from this variety of the soy bean was first treated with petroleum benzine and obtained nearly free from fat. By extracting 2000 grams of the flour, thus prepared, with brine and proceeding in exactly the same manner as in making preparation 1 from the yellow soy bean, a large precipitate of globulin was obtained, a part of which, when washed and dried, gave preparation 14, weighing twenty-five grams. The remainder of the globulin was drained on filters, suspended in water and 300 cc. of ten per cent. sodium chloride solution added, bringing the volume to 1000 cc. Under this treatment very nearly all dissolved. An equal volume of water was then added to the solution and the separated globulin allowed to settle. The precipitate was collected on a filter and redissolved in ten per cent. brine. The solution was filtered perfectly clear and dialyzed for forty-eight hours. The precipitate, so separated, was washed with water and with alcohol and dried, giving preparation 15, weighing 83.5 grams. The solution filtered from the first precipitation of 15, caused by dilution, as just described, was cooled to 8° during twenty-four hours. The substance thus separated, which formed a clear semifluid layer at the bottom of the jar, was thoroughly washed with water and with alcohol and dried. It weighed 12.85 grams and formed preparation 16.

After drying at 110° to constant weight these preparations were analyzed as follows:

GLYCININ.

	14	15	16	Average.
Carbon	51.80	52.15	52.09	52.01
Hydrogen	6.93	6.87	6.86	6.89
Nitrogen	17.45	17.48	17.47	17.47
Sulphur	0.75	0.72	0.70	0.72
Oxygen	23.07	22.78	22.88	22.91
	100.00	100.00	100.00	100.00
Ash	0.39	0.42	0.28	

These results agree well with those obtained from the yellow soy bean.

GLYCININ.

	Yellow Soy bean.	*Kiyusuki diadzu* Soy bean.
Carbon	51.85	52.01
Hydrogen	6.92	6.89
Nitrogen	17.45	17.47
Sulphur	0.72	0.72
Oxygen	23.06	22.91
	100.00	100.00

CONCLUSION.

The soy bean contains as its chief proteid constituent *glycinin*, a globulin similar in properties to legumin, but of somewhat different composition, containing nearly twice as much sulphur, four-tenths per cent. more carbon, and one-half per cent. less nitrogen.

We give as the composition of this proteid the results of our analysis of preparation 9, which was obtained from a neutral and perfectly clear extract, for we believe that this represents more accurately the true composition of glycinin than the average of all the preparations.

GLYCININ.

Carbon	52.12
Hydrogen	6.93
Nitrogen	17.53
Sulphur	0.79
Oxygen	22.63
	100.00

The soy bean contains a more soluble globulin which resembles phaseolin in composition, and, so far as we could ascertain, also in its reactions. The amount of this proteid is small and the evidence that it is in reality phaseolin was not wholly satisfactory.

Besides these globulins about one and five-tenths per cent. of the albumin-like proteid *legumelin* was obtained. We have found legumelin in a number of other leguminous seeds, the pea, vetch, horse bean, lentil, adzuki bean, and cow pea. The properties of legumelin are given in our papers on "The Proteids of the Pea, Lentil, Horse Bean, and Vetch."[1] The composition of legumelin as found in the soy bean is as follows:

[1] This Journal, 20, 410.

LEGUMELIN.

Carbon	53.06
Hydrogen	6.94
Nitrogen	16.14
Sulphur	1.17
Oxygen	22.69
	100.00

A small quantity of proteose was also obtained from the soy bean having the following composition:

PROTEOSE.

Carbon	48.76
Hydrogen	6.28
Nitrogen	16.14
Sulphur } Oxygen }	28.82
	100.00

Owing to the small amount of proteose no evidence was obtained as to the purity or individuality of this preparation.

ELECTRICAL DISTURBANCE IN WEIGHING.

BY H. K. MILLER.

Received February 28, 1898.

WHILE making some fat determinations, I found a practice, which I believe is quite common among chemists, to be the source of quite serious errors. The practice is that of wiping a flask with a dry cloth just previous to weighing it. In making a second weighing of a flask containing an oil which had been extracted from a sample, I was very much surprised to find a considerable increase over the weight first obtained.

The first idea that presented itself was that the oil had suffered oxidation during the second heating, but this idea was dispelled when to my greater surprise a third weighing showed the flask and contents to weigh even less than at first. Careful experiments led to the conclusion that in wiping the flask it became electrified, and that this static charge, acting on the floor of the balance, induced on it a charge of opposite character, and that the mutual attraction between these two charges of electricity had the effect of apparently increasing the weight of the flask.