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PROTEIDS OF THE HORSE BEAN (*Vicia faba*).[1]

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THE only references which we have found to investigations of the proteids of this seed that at present have any importance are contained in papers by Ritthausen.[2]

In his earlier work[3] Ritthausen described as legumin the proteid extracted from the horse bean by potash water. Later[4], he gave the results of treating the earlier preparations with salt solution and also of extracting the seeds with brine and precipitating the dissolved proteid by dilution or by acids, and concluded that the preparations which he obtained were mixtures of two proteids which could only be separated by dissolving in acid or alkali, precipitating by neutralization and extracting the precipitate with brine; the part dissolving he regarded as conglutin; that undissolved as legumin. A careful study of Ritthausen's work leaves us in much doubt respecting the nature of the proteids of this seed, and we have therefore undertaken the present investigation.

In order to avoid contaminating our preparations with tannin, which Ritthausen found to be present in considerable quan-

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

[2] Early references to legumin have been already noticed in our paper on "Legumin of the Pea and Vetch," Report of this Station for 1895, p. 262. This Journal, 18, 583.

[3] Die Eiweisskorper, etc., Bonn, 1872, p. 170.

[4] *J. prakt. Chem.*, 26, 504, and 29, 448.

tity in the skin of these beans, we removed the greater part of the outer coating from the coarsely broken seeds by a current of air and the remainder by hand-picking. In this way it was possible to separate the brown outer seed-coat completely and by then grinding the broken beans to obtain a flour free from tannin.

A preliminary extraction made with this flour gave us a large yield of proteid which, in general, had nearly the properties and composition of the products similarly obtained from the pea and lentil, but differed throughout in containing less carbon than the corresponding substances from these other seeds, and in being, to a considerable extent, soluble in water. As these aqueous solutions reacted strongly acid with litmus and when neutralized gave precipitates soluble in brine which were reprecipitated by dilution, we were led to believe that the differences which we had found between the proteids of this seed and those of the pea, lentil, and vetch were due to a combination of the proteids with the acid of the seed. As this view appeared to be confirmed, we omit further details of our first extraction and proceed to an account of the second.

Two kilograms of the bean flour were treated with ten liters of ten per cent. salt solution, protected with thymol, and left over night in a cool place. The extract was strained through fine bolting-cloth and allowed to deposit suspended matter during two hours. The turbid liquid was siphoned off, centrifugated, and filtered nearly clear. The extract was then saturated with ammonium sulphate, the precipitate produced filtered out, removed from the paper, suspended in a little water and dialyzed for twenty-four hours, whereby so much of the sulphate was removed that the proteid dissolved. The solution so obtained was then filtered perfectly clear through a pulp filter and dialyzed for forty-four hours. The precipitate which had formed at first separated in spheroids, but these on settling united to a coherent mass, A, from which the solution B was decanted.

A portion of the precipitate A, weighing twenty-eight grams when air-dry, was washed thoroughly with water (in which it partly dissolved) and then with alcohol and dried at 110° for analysis.

LEGUMIN, 76.[1]

Carbon	51.55
Hydrogen	6.91
Nitrogen	18.12
Sulphur	0.48
Oxygen	22.94
	100.00
Ash	0.54

The remainder of precipitate A was dissolved in brine and the resulting solution, having an acid reaction, was neutralized to litmus by adding very dilute potash water. This required 0.36 gram of potassium hydroxide. The slightly turbid solution was filtered absolutely clear and dialyzed for forty-eight hours. The large precipitate which resulted was found to dissolve completely in brine and to yield a perfectly neutral solution, which, when heated in a boiling water-bath, gave some coagulum. A portion of this precipitate was washed with water and alcohol, dried at 110°, and analyzed.

LEGUMIN, 77.

Carbon	51.87
Hydrogen	6.97
Nitrogen	18.05
Sulphur	0.38
Oxygen	22.73
	100.00
Ash	0.84

The remainder of this substance was dissolved in 250 cc. of three per cent. brine and the solution diluted to 600 cc. A rapidly settling precipitate resulted which formed a fluid deposit from which the somewhat turbid mother-liquor was soon decanted; the proteid was washed thoroughly with water and alcohol, yielding when dry 19.13 grams of preparation 78.

LEGUMIN, 78.

Carbon	51.79
Hydrogen	7.06
Nitrogen	18.10
Sulphur	0.40
Oxygen	22.65
	100.00
Ash	0.79

[1] Numbered consecutively with the preparations of the proteids of the lentil. This Journal, **20**, 362.

The solution decanted from 78 was diluted with 150 cc. of water and a second precipitate obtained, wholly like the first, which when dried weighed eleven grams and had the following composition :

LEGUMIN, 79.

Carbon	51.90
Hydrogen	6.94
Nitrogen	18.12
Sulphur	0.38
Oxygen	22.66
	100.00
Ash	0.59

The solution from which 79 had separated was further diluted with 500 cc. of water and allowed to stand over night in a cool room. The next morning the solution was decanted from a small precipitate that had settled out. This, after drying, weighed five grams and had the following composition :

LEGUMIN, 80.

Carbon	51.92
Hydrogen	7.04
Nitrogen	18.11
Sulphur	0.30
Oxygen	22.63
	100.00
Ash	0.60

The solution decanted from 80 was dialyzed for four days but only a trace of proteid separated. Preparations 78, 79, and 80 contained no trace of coagulable proteid and may therefore be considered to be pure legumin. Since, however, 77, of which these were fractions, was shown by this treatment to contain only a very little coagulable matter, it too is essentially pure legumin as indicated by the analysis.

Solution B, described on page 394, was further dialyzed for twenty-four hours and the resulting precipitate C filtered out and the filtrate D treated as described on page 397.

The precipitate C was dissolved in ten per cent. brine, and the resulting solution diluted until it contained one per cent. of salt. The precipitate that formed was filtered out and the filtrate added to solution D. The precipitate was again dissolved in

salt solution, filtered clear, exactly neutralized with dilute potash water, and dialyzed for forty-eight hours.

The globulin which had separated was filtered out. The filtrate contained a little more proteid, which yielded a coagulum on heating in a boiling water-bath.

The globulin was dissolved in 200 cc. of two per cent. brine, and the solution diluted with 200 cc. of water, which threw down a precipitate from which the solution E was decanted and treated as further described, while the precipitate was redissolved in salt solution, which was then dialyzed for twenty-four hours.

The precipitate which finally resulted, after washing and drying, weighed sixteen grams, and when dried at 110° had the following composition :

GLOBULIN, 81.

Carbon	51.89
Hydrogen	7.03
Nitrogen	17.67
Sulphur	0.34
Oxygen	23.07
	100.00
Ash	0.76

The solution filtered from 81 contained but little proteid, which coagulated on heating to 99°.

Solution E, on diluting with an equal volume of water, gave a precipitate that, when washed and dried, weighed 4.74 grams, having the following composition :

GLOBULIN, 82.

Carbon	52.31
Hydrogen	7.04
Nitrogen	17.70
Sulphur	0.21
Oxygen	22.74
	100.00
Ash	0.57

Solution D, noted on page 396, being inconveniently bulky, was saturated with ammonium sulphate, and the precipitate thus separated was dissolved in a little water; the solution was filtered clear, and dialyzed for five days. The deposited substance was

filtered out and the filtrate F treated as described later. The precipitate was dissolved in brine, the solution obtained was filtered clear and dialyzed for three days whereby all but a trace of the proteid was precipitated. This was filtered out, dissolved in 100 cc. of two per cent. salt solution and precipitated by adding fifty cc. of water. After the proteid, thus thrown down, had settled, the supernatant liquid was decanted and fifty cc. more water added, which caused a second precipitation. The solution from which this had separated was then dialyzed and the globulin contained in it precipitated. Thus three successive fractions were obtained which, when washed and dried, weighed respectively 5.65, 3.87, and 2.73 grams. When dried at 110° and analyzed these were found to have the following composition:

VICILIN.

	83	84	85
Carbon	52.53	52.53	52.40
Hydrogen	6.93	6.98	7.09
Nitrogen	17.18	17.61	17.54
Sulphur	0.19	0.10	0.13
Oxygen	23.07	22.78	22.84
	100.00	100.00	100.00
Ash	0.26	0.18	0.13

Filtrate F, from which the substance yielding the three preceding preparations had been derived, was further dialyzed for seven days until freed from sulphates, which caused the separation of a small precipitate that, when filtered out, washed, and dried, weighed 1.64 grams and gave the following results on analysis:

PROTEID, 86.

Carbon	52.43
Hydrogen	6.87
Nitrogen	16.42
Sulphur, Oxygen	24.28
	100.00
Ash	0.28

The filtrate from 86 was then heated to just 60° for about two hours, which caused a coagulum that was filtered out, washed

with hot water and with alcohol, dried, and found to weigh 1.60 grams. The filtrate from this coagulum was heated for some time at 75° and a second coagulum obtained, weighing 1.80 grams. Dried at 110°, the two preparations had the following composition:

LEGUMELIN.

	87	88
Carbon	52.81	52.98
Hydrogen	6.98	6.89
Nitrogen	16.49	16.43
Sulphur	23.72	1.32
Oxygen		22.38
	100.00	100.00
Ash	0.23	0.33

These two preparations are probably legumelin, although their carbon is distinctly less than that found in our preparations from other leguminous seeds. Since the solution from which these preparations separated had not been previously neutralized, we are inclined to ascribe this difference to an acid combined with the proteid.

The filtrate from 88 was next dialyzed against alcohol until all proteid matter was precipitated. This was filtered out, washed with absolute alcohol, dried, and found to weigh 6.78 grams. This preparation was dissolved in a little water, filtered from a slight insoluble residue; the solution was dialyzed for five days, then heated to boiling, and filtered from a trace of coagulum. The filtrate, which gave no precipitate on saturating with salt, was then concentrated to small volume over a water-bath and precipitated by pouring into alcohol. In this way 4.45 grams of proteose was obtained, which, when dried at 110° and analyzed, was found to have the following composition:

PROTEOSE, 89.

Carbon	50.24
Hydrogen	6.66
Nitrogen	17.11
Sulphur	1.87
Oxygen	24.12
	100.00
Ash	0.48

In order to confirm the foregoing results another extraction was made by treating 1200 grams of bean meal with 4200 cc. of water containing 9.44 grams of baryta, just enough to saturate the acid of the seed, using litmus as an indicator. This amount was ascertained by a careful preliminary test with 100 grams of the meal using a carefully standardized solution of baryta. After thoroughly mixing the meal with the water, an equal volume of ten per cent. brine was added, causing the gummy meal residue to separate in masses which settled quite rapidly and left the solution comparatively clear.

The residue was then strained out on fine bolting-cloth and the extract allowed to stand a short time, when it was siphoned from the sediment, filtered nearly clear, and saturated with ammonium sulphate. The precipitate produced was filtered out, suspended in water, and dialyzed over night. The next morning the solution of the proteid, which had resulted on dialyzing away the greater part of the adherent sulphate, was filtered and dialyzed for four days. A large precipitate of globulin separated which was filtered out and the filtrate G was treated as is subsequently described. The precipitate was redissolved in brine, filtered perfectly clear, and dialyzed for forty-two hours, whereby a large amount of globulin separated in form of spheroids that united on settling to a coherent mass. From this the solution H was decanted almost completely. The precipitate was dissolved in 500 cc. of two per cent. salt solution and the globulin again thrown down by diluting the salt solution to 1.33 per cent. After the large precipitate produced had settled, the solution I was decanted and the globulin redissolved in 100 cc. of ten per cent. brine and precipitated by diluting to 750 cc. As the substance still contained some coagulable matter it was redissolved four times in fifty cc. of ten per cent. brine and precipitated by diluting to 500 cc. The final precipitate gave only an opalescence on heating its solution in a boiling water-bath. Washed with water and with alcohol and dried over sulphuric acid, this preparation weighed forty-one grams, and had the following composition when dried at 110°:

LEGUMIN, 90.

Carbon	51.55
Hydrogen	7.03
Nitrogen	17.95
Sulphur	0.38
Oxygen	23.09
	100.00
Ash	0.15

Solutions G, H, I, and those from the four last precipitations of 90 were separately dialyzed and the globulin which deposited was all dissolved together by adding fifty cc. of ten per cent. brine to water in which the substance was suspended. The resulting solution contained one and seven-tenths per cent. of salt and was diluted to a content of one and three-tenths per cent. This gave a precipitate on which the same treatment was repeated. The two solutions decanted from these precipitations were united and diluted with 200 cc. of water, the precipitate was united with the twice precipitated proteid just described and the solution J set aside for further treatment. The united precipitates were dissolved with fifty cc. of ten per cent. salt solution, and water added to 400 cc. The precipitate produced, which still yielded some opalescence in the boiling water-bath, was washed with water and alcohol, and, when dried, weighed 15.93 grams, and had the following composition :

LEGUMIN, 91.

Carbon	51.72
Hydrogen	6.95
Nitrogen	18.00
Sulphur	0.42
Oxygen	22.91
	100.00
Ash	0.18

The solution decanted from 91 was united with solution J, before noticed, and diluted until the salt content was one per cent. After standing over night, the solution was decanted from the precipitate, which when washed and dried over sulphuric acid weighed nine and eight-tenths grams, and when dried at 110° had the following composition :

LEGUMIN, 92.

Carbon	51.70
Hydrogen	7.12
Nitrogen	18.05
Sulphur	0.36
Oxygen	22.77
	100.00
Ash	0.16

The solution decanted from 92 was dialyzed free from chlorides, the precipitate produced dissolved in one per cent. brine, and the solution diluted with an equal volume of water. After the proteid which separated had settled out, the solution was decanted and the deposit was washed with water and alcohol. When dry 14.41 grams of proteid were obtained, which were analyzed with the following results:

GLOBULIN, 93.

Carbon	52.14
Hydrogen	7.08
Nitrogen	17.59
Sulphur	0.15
Oxygen	23.04
	100.00
Ash	0.13

By adding a quantity of water to the solution decanted from 93 the globulin remaining in solution was precipitated and gave, when dried, 5.80 grams of proteid of the following composition:

VICILIN, 94.

Carbon	52.36
Hydrogen	7.12
Nitrogen	17.43
Sulphur	0.10
Oxygen	22.99
	100.00
Ash	0.10

Solution G, page 400, was saturated with ammonium sulphate, and the proteid thereby precipitated was dissolved in water, the solution filtered perfectly clear and dialyzed six days, when the precipitate produced was filtered out, washed, and treated as usual, giving preparation 95, weighing 4.23 grams.

PROTEID, 95.

Carbon	52.21
Hydrogen	7.12
Nitrogen	16.50
Sulphur	1.23
Oxygen	22.94
	100.00
Ash	0.33

The filtrate from 95 was then heated to 65° in a water-bath, the coagulum filtered out, and the filtrate heated to 85°, which caused a second coagulum. The two preparations, 96 and 97, were washed with hot water, dehydrated with absolute alcohol, dried, and found to weigh 3.47 grams and 1.38 grams, respectively.

LEGUMELIN.

	96	97
Carbon	52.68	53.29
Hydrogen	7.14	7.05
Nitrogen	15.96	16.00
Sulphur	1.33	1.24
Oxygen	22.89	22.42
	100.00	100.00
Ash	0.25	0.31

The filtrate from 97 gave no coagulum on boiling and was therefore saturated with salt, but as no precipitate formed, salt saturated acetic acid was added as long as proteid was thereby separated. The resulting precipitate was dissolved in water, filtered clear, and dialyzed till free from chlorides. The salt saturated filtrate was neutralized with sodium carbonate and also dialyzed till free from chlorides. Both solutions were next dialyzed in alcohol until concentrated to small volume, then were treated with excess of alcohol, and the precipitates obtained were filtered out. That from the salt saturated solution amounted to hardly more than a trace, while that from the acetic acid precipitate 98 weighed 4.14 grams.

PROTOPROTEOSE, 98.

Carbon	49.96
Hydrogen	6.76
Nitrogen	16.95
Sulphur	2.75
Oxygen	23.58
	100.00
Ash	0.28

The large amount of sulphur found in this preparation was confirmed by a second closely agreeing determination. The small quantity of ash and the method of preparation make it impossible that this sulphur could be due to admixture of either calcium sulphate or ammonium sulphate.

In the horse bean, as in the pea and lentil, the proteids soluble in salt solution are legumin, vicilin, legumelin, and proteose. In the following tables the analyses of the purer preparations of these proteids may be readily compared:

LEGUMIN.

	76	77	78	79	80	90	91	92	Aver.
Carbon	51.55	51.87	51.79	51.90	51.92	51.55	51.47	51.70	51.72
Hydrogen	6.91	6.97	7.06	6.94	7.04	7.03	7.02	7.12	7.01
Nitrogen	18.12	18.05	18.10	18.12	18.11	17.95	18.00	18.05	18.06
Sulphur	0.48	0.38	0.40	0.38	0.30	0.38	0.42	0.36	0.39
Oxygen	22.94	22.73	22.65	22.66	22.63	23.09	23.09	22.77	22.82
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

VICILIN.

	82	83	84	85	93	94	Aver.
Carbon	52.31	52.53	52.53	52.40	52.14	52.36	52.38
Hydrogen	7.04	6.93	6.98	7.09	7.08	7.12	7.04
Nitrogen	17.70	17.28	17.61	17.54	17.59	17.43	17.52
Sulphur	0.21	0.19	0.10	0.13	0.15	0.13	0.15
Oxygen	22.74	23.07	22.78	22.84	23.04	22.99	22.91
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

LEGUMELIN.

	87	88	96	97	Aver.
Carbon	52.81	52.98	52.68	53.29	52.94
Hydrogen	6.98	6.98	7.14	7.05	7.02
Nitrogen	16.49	16.43	15.96	16.00	16.22
Sulphur	23.72	1.32	1.33	1.24	1.30
Oxygen		22.38	22.89	22.42	22.52
	100.00	100.00	100.00	100.00	100.00

PROTEOSE.

	89	98
Carbon	50.24	49.95
Hydrogen	6.66	6.76
Nitrogen	17.11	16.95
Sulphur	1.87	2.75
Oxygen	24.12	23.58
	100.00	100.00

In order to determine the amount of the proteids of this seed extracted by water, 200 grams of the fine meal were thoroughly mixed with 2000 cc. of water so that all lumps were broken up, and the coarse residue was strained out on bolting-cloth. After standing two hours, the greater part of the starch, etc., settled, and 1400 cc. of extract were siphoned off and filtered perfectly clear on a pulp filter, the first 300 cc. being rejected and the next 1000 cc. collected separately. The latter was dialyzed for four days, in which time all the proteid separable in this way deposited as spheroids. The precipitate, washed and dried, was equal to sixteen per cent. of the meal.

The process just described was exactly repeated save that enough baryta was at first added to make the extract neutral to litmus: the yield of proteid was eighteen per cent.

The proteid from the first extraction contained 1.16 per cent. of ash and, reckoned ash-free, 17.50 per cent. of nitrogen, showing it to be nearly pure globulin, while that from the second, neutralized extract, was less pure, as it contained 5.41 per cent. of ash and contained, ash-free, 16.96 per cent. of nitrogen. The reactions of these extracts were compared under identical conditions with the following result:

Both extracts, like those from the pea, lentil, and vetch, when poured into water gave, where the two fluids came in contact, a slight cloud which disappeared on shaking. Unlike the neutralized extract, as well as unlike both neutral and acid extracts of the other legumes, the unneutralized extract of the horse bean gave a heavy precipitate with sodium chloride, even with perfectly pure salt which we prepared from *pure* soda and *pure* hydrochloric acid and proved to be absolutely neutral. This precipitation of the globulin would indicate the presence of an acid compound, but the extract gave but a very slight precipitate on neutralizing with baryta, probably due to phosphates. The addition of acetic acid gave a large precipitate soluble in dilute brine, as did calcium chloride and calcium sulphate.