# **PROTEIDS OF THE VETCH.**<sup>1</sup>

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL. Received March 29, 1898.

IN order to further fractionate vetch globulin, we treated a quantity of preparation 17<sup>2</sup> with 900 cc. of three per cent. brine and, after filtering out the insoluble residue, we added an equal volume of water to the clear filtrate, and then cooled the mixture to 5°. The precipitate thus caused was washed and dried, forming preparation 99,3 and the filtrate was diluted with an equal volume of water, which caused a second precipitate, preparation 100. The residue, insoluble in the three per cent. brine, had become swollen and gelatinous, as has always been observed with the insoluble form of legunin, so that it stopped the pores of the filter. It was therefore washed by decantation with salt solution and finally with water. As the salt was washed out the substance lost its gelatinous properties and became granular and easily filtered. It was next treated with one per cent. sodium carbonate solution, and the small portion insoluble therein was filtered out. The solution was partly neutralized with hydrochloric acid, and carbonic acid gas passed through it, which precipitated nearly all the dissolved proteid. This was filtered out, dissolved in brine, and the clear solution dialyzed. The substance thus separated, when washed and dried, formed preparation 101.

These three preparations were carefully analyzed with the following results :

LEGUMIN. 100 101 99 Carbon ..... 51.72 51.52 51.79 Hydrogen ..... 6.97 6.95 7.03 Nitrogen..... 18.13 18.08 18.03 Sulphur .... 0.43] 23.47 23.15 Oxygen ..... 22.75 100.00 100.00 100.00

<sup>1</sup> Reprinted from advance sheets of the Report of the Connecticut Agricultural Experiment Station for 1897. Communicated by the authors.

<sup>2</sup> Annual Report of the Connecticut Agricultural Experiment Station for 1895, p. 277. This Journal, 18, 598.

<sup>8</sup> Numbered consecutively with the preparations of the proteids of the horse bean.

Another extraction was made by treating 500 grams of vetch meal with water and then with ten per cent. sodium chloride solution. After filtering clear, the salt extract was saturated with ammonium sulphate, the precipitate produced was dissolved in brine, the solution filtered perfectly clear, and dialyzed. In this way thirty-five grams or seven per cent. of the meal was obtained as preparation 102, having the following composition:

### LEGUMIN, 102.

Carbon 51.62
Hydrogen 6.92
Nitrogen 17.88
Sulphur 0.50
Oxygen 23.08
00.001
Ash 0.23

Again, a quantity of vetch meal was digested in saturated salt solution and the dissolved legumin precipitated by dialysis. After drying at 110° this gave the following figures :

# LEGUMIN, 103.

Carbon 51.86
Hydrogen 6.99
Nitrogen 17.97
Sulphur
Oxygen
100.00
Ash 0.57

Another extraction was made by treating 1200 grams of meal with three liters of water containing enough baryta to exactly neutralize the acid of the seed when tested with litmus paper. After thoroughly mixing, three liters of ten per cent. salt solution were added and the residual meal strained out on fine bolting-cloth. After standing a short time the greater part of the starch settled and the liquid was siphoned off, filtered perfectly clear, and saturated with ammonium sulphate. The precipitate produced was filtered out, suspended in water, and dialyzed over night, whereupon the proteid dissolved. Its solution was filtered clear and dialyzed for two days with separation of a large precipitate.

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This was filtered out and the filtrate A examined as will be described later. The precipitate was dissolved in brine, the solution filtered perfectly clear from a small amount of insoluble matter, and dialyzed for two days. The large coherent deposit was washed with water and with alcohol, and dried over sulphuric acid. This preparation, 104, weighed seventy-five grams and, as it was obtained from nearly three-fourths of the total extract, represented about ten per cent. of the meal. When dried at 110° and analyzed, the following results were obtained :

### LEGUMIN, 104.

Carbon	51.59
Hydrogen	7.07
Nitrogen	18.03
Sulphur	o.36
Oxygen	<b>2</b> 2.95
-	
1	00,00
Ash	0.15

The filtrate A was returned to the dialyzer and, after three days, filtered from a small precipitate which, washed and dried, weighed six and a half grams and gave preparation 105, having the following composition :

PROTEID, 105.
Carbon 51.81
Hydrogen 7.10
Nitrogen 17.07
Sulphur 0.57
Oxygen 23.45
100.00
Ash 0.38

The filtrate from 105 was saturated with ammonium sulphate and the precipitate produced filtered out, dissolved in as small a quantity of water as possible, the solution filtered clear, and dialyzed for seven days, when it was filtered from a small precipitate that was partly soluble in salt solution. This was washed with water and alcohol and formed preparation 106.

The filtrate from 106 was heated to  $65^{\circ}$  in a large water-bath and a coagulum obtained which, when filtered out, washed, and dried, formed preparation 107, weighing three and two-tenths grams. The filtrate from this, on heating to  $85^{\circ}$ , gave a small coagulum that, when washed and dried, weighed only 0.88 gram, 108.

LEGU	MELIN	•
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I	:06	107	108
Carbon 53	3.05	53.33	••••
Hydrogen 7	.29	6.93	• • • •
Nitrogen 16	5.25	16.10	16.16
Sulphur 1	.19	0.85	••••
Oxygen 22	2.22	22.79	••••
		———	
IO	0.00	100.00	••••
Ash c	.39	0.26	0.38

The filtrate from 108 gave no coagulum on boiling. It was concentrated, partially by evaporation and further by dialysis in alcohol. A small precipitate thus separated, which was dehydrated with absolute alcohol and dried, giving one gram of preparation 109, that contained 12.74 per cent. of ash and, reckoned ash-free, 15.71 per cent. of nitrogen.

The results show that the salt extracts of the vetch seed contain no other proteids than legumin, legumelin, and a very small proportion of proteose. Vicilin, which was found in the pea, horse bean, and lentil, is not present in detectable quantity in the vetch.

Our new analyses of these proteids are tabulated as follows :

								ormer
	99	100	101	102	103	104	Aver.	Aver.
Carbon	51.72	51.52	51.79	51.62	51.86	51.59	51.69	52.09
Hydrogen · · ·	6.97	6.95	7.03	6.92	6.99	7.07	6.99	6.88
Nitrogen	18.13	18.08	18.03	17.88	17.97	18.03	18.02	18.0 <b>2</b>
Sulphur	0.43 \	23.45	23.15	0.50	23.18	0.36	0.43	0.46
Oxygen	ر 22.75 <sup>(</sup>	23.45	23.15	23.08	23.10	22.95	22.87	22.55
-	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
LEGUMELIN.								
			20	106	107	108	Aver	•
Carbon	••••	••••• 5	3.55	53.05	53.33		53.31	:
Hydrog	en	(	5.70	7.29	6.93		6.97	7
Nitroge	n	16	5.46	16.25	16.10	16.16	16.24	ŀ
Sulphu	r		1.02	1.19		••••	1.11	[
Oxygen	•••••	••••• 2	2.27	22.22		••••	22.37	7
		100	D.00 I	00.00			100.00	- >

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#### PROTEOSE, 22.

Carbon 50.85 Hydrogen 6.75
Nitrogen 16.65
Sulphur
Oxygen
100.00

# THE PROTEIDS OF THE PEA, LENTIL, HORSE BEAN, AND VETCH.<sup>1</sup>

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL. Received March 29, 1898.

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 twothirds 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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