almost completely precipitated by saturating with sodium chloride. It is not at all impossible that this change, too, may have been caused by acid, for these preparations stood for several years in the laboratory, the air of which at times contained some acid vapors. We thus see the same change taking place in the dry proteid on long keeping as those definitely caused by minute quantities of acid.

That this change to a condition in which the globulin is precipitated by salt is an intermediate step towards the formation of the insoluble form, the so-called "albuminate" of Weyl is evident from what has already been stated, especially the fact that by treatment with warm salt solution this insoluble matter can be changed into the form soluble in cold salt solution and precipitable by saturation with salt.

In this connection it is interesting to note that the only animal globulin obtained from an acid tissue is myosin, and that this myosin not only is readily precipitated by saturating with salt, but quickly and spontaneously changes to the insoluble form known as syntonin. In the dead muscle the amount of acid greatly exceeds that used in our experiments, for its presence is plainly shown by the strong acid reaction of the muscle serum. In alkaline muscle plasma myosin is not found, but myosinogen, paramyosinogen, and myoglobulin. The last three are described as precipitated by saturation with sodium chloride, but it may be that when tested in this respect the formation of acid had already begun and had reached a point where it caused precipitation with salt, but could not be detected by the usual tests.

THE PROTEIDS OF THE SUNFLOWER SEED.[1]

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

Received April 12, 1897.

THE only published observations on the proteid of the sunflower seed which we have found were made by Ritthausen[2] and by Vines.[3] By extracting with very dilute alkali Ritthausen obtained from finely ground oil-free meal 44.71 per cent. of proteid, having the composition given under 1. By treating with sodium chloride brine, diluting the extract with

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

[2] Pflüger's Archiv., 21, 89, 1880.

[3] *J. Physiol.*, 3, 93.

five volumes of water and passing carbon dioxide through the solution he got 25.3 per cent. with the composition given under 2, and by exhausting a preparation obtained in the same manner as 1 with brine, and proceeding as with 2, he got a preparation whose analysis is given under 3.

SUNFLOWER PROTEID.	RITTHAUSEN.		
	1.	2.	3.
Carbon	51.88	51.51	51.18
Hydrogen	6.66	6.76	6.82
Nitrogen	17.99	18.21	18.06
Sulphur	0.71	0.61	
Oxygen	22.76	22.91	23.94
	100.00	100.00	100.00

Vines states that if a section of sunflower seed be treated with ether to remove oil it will be found that the aleurone grains, though readily soluble in ten per cent. sodium chloride solution, will not dissolve in saturated solution; if, however, they be treated with alcohol instead of ether, the globulin of which these grains consist behaves like vitellin, that is, it dissolves in a saturated solution of sodium chloride.

Vines further states that "it is of interest to note the fact that most of the substances which I found in the grain recur in the crystalloids, more especially vitellin and its derivatives; thus the peculiar globulin which forms the crystalloids of Ricinus appears to be in the grains of Helianthus."

Ritthausen's results indicate that by far the greater part of the proteid matter of the sunflower seed has a uniform composition, and that a large part of this proteid is insoluble in salt solution, but soluble in dilute alkali. The composition which he found for this proteid resembles that of the globulin edestin, which we have found in many seeds, the only difference being a slightly lower content of nitrogen. On this account it seemed to us desirable to examine this proteid and determine its relation to edestin. This was the more important, because Vines' statement of its behavior when treated with ether and with alcohol showed it to possess the same peculiar relations to salt solutions observed by one of us[1] in studying the globulin of the castor bean, *Ricinus communis*. As the deportment of globulins to saturated sodium chloride solutions has been made the basis of

[1] Osborne: Report Conn. Expt. Station, 1892, p. 138, and *Am. Chem. J.*, 14, 671.

a division of these bodies into two main classes, it is important for us to know whether this is founded on fundamental differences in the proteids, or is simply due to the unlike conditions under which the proteids are found.

Sunflower seeds were crushed and a large part of the woody shells removed. The meal was then ground under benzine and after freeing from oil, air-dried. This meal when treated with ten per cent. sodium chloride brine yielded an extract of a strong blackish green color, from which a considerable quantity of proteid could be separated by dilution, by dialysis, or by saturation with sodium chloride.

When heated, this extract becomes turbid at 48° and flocks separated at 62°. The solution heated to 75° and filtered from this slight coagulum yielded a large precipitate when saturated with salt, thus showing that most of the substance thus precipitated is not, as *myosin* is said to be, coagulated below 75°.

The unheated extract saturated with sodium chloride gives a precipitate which when dissolved in ten per cent. brine coagulates at the same temperature as the original extract, but the amount of this coagulum is but a small fraction of the substance precipitated by saturation with salt.

The following preparations were made, but as subsequently pointed out, were found to be more or less impure, so that these results have value only as affording evidence of the uniform composition of the globulin extracted by salt solution from the sunflower seed.

	1	2	3	4	5
Carbon	51.57	51.77	51.65	51.69	51.85
Hydrogen	6.81	6.83	6.72	6.80	6.84
Nitrogen	18.16	18.20	18.17	18.24	18.00
Sulphur } Oxygen }	23.46	23.20	23.46	0.78 21.49	23.21
	100.00	100.00	100.00	100.00	100.00

	6	7	8	9
Nitrogen	18.20	18.23	18.09	18.07

Of these, 1 is the total globulin extracted by brine from one portion of oil-free meal; 2, 3, 4, and 5 are fractional precipitates from another similar extraction; 6, substance precipitated by saturating the salt extract with sodium chloride; 7 and 8, substance soluble in saturated sodium chloride solution; and 9, that

precipitated by cooling an extract made with a one and a half per cent. salt solution heated to 60°.

These results show that the most abundant proteid of the sunflower seed consists of a single globulin, and that the proteid precipitated by saturating with sodium chloride contains the same amount of nitrogen as the proteid soluble in a saturated solution of this salt. As Vines stated that the substance of the aleurone grains was soluble in a saturated salt solution after treatment with alcohol, while after treatment with ether it was insoluble therein, although soluble in ten per cent. salt solution, we thought that possibly by treating our meal with alcohol we might remove some substance, perhaps an acid soluble in alcohol, but insoluble in ether, which might be the cause of this peculiar behavior of the proteid. We accordingly extracted a quantity of sunflower meal with alcohol of 0.820 sp. gr., and in order to determine whether acid had been removed we attempted to titrate a portion of the extract with a one per cent. solution of potash. On adding the alkali a colored precipitate resulted, which rendered the indicator (*phenolphthalein*) useless. The attempt was then repeated, omitting the indicator. When the potash solution was added a bright chrome yellow color resulted which gradually increased with the formation of a precipitate as the quantity of the potash was increased. With a larger excess of potash the precipitate redissolved. This reaction we found to be due to helianthotannic acid.[1] The results of our investigation of this acid will be given in another paper.

Having now found a very delicate test for this acid, we applied it to our preparations of globulin and obtained a strong reaction in every case. It was therefore necessary to remove this acid from the meal before attempting to obtain the proteid and accordingly the extraction of the meal with alcohol was continued. It was, however, practically impossible to remove the acid so completely as to obtain no yellow reaction when the extract was treated with potash.

The meal which had been nearly freed from this acid was washed with ether and air-dried. 100 grams were extracted with ten per cent. sodium chloride brine and the filtered extract saturated with salt. An abundant precipitate separated, just as

[1] Ludwig and Kromayer, N. Br., 99, 1, 285.

with meal which had not been treated with alcohol. This was filtered off, dissolved in ten per cent. brine and again precipitated by saturation with salt. This precipitate was again dissolved in salt solution, filtered perfectly clear, and dialyzed. The globulin which was thus precipitated was filtered out, washed with water and alcohol and dried over sulphuric acid. This preparation, 10, weighed seven and four-tenths grams and had the following composition :

SUNFLOWER GLOBULIN, PREPARATION 10.

Carbon	51.27
Hydrogen	6.55
Nitrogen	18.21
Sulphur	0.78
Oxygen	23.25
	100.00
Ash	0.31

The saturated sodium chloride solutions filtered from the two precipitations of 10, were united and dialyzed until free from chloride; the resulting precipitate was filtered out and treated as 10 had been. Preparation 12 was thus obtained, which on analysis gave the following results :

SUNFLOWER GLOBULIN, PREPARATION 12.

Carbon	51.58
Hydrogen	6.55
Nitrogen	18.29
Sulphur	0.97
Oxygen	22.61
	100.00
Ash	0.29

As both the preceding preparations were found to contain detectable quantities of helianthotannic acid another attempt was made to prepare some meal which should be practically free from this acid.

One hundred grams of meal were therefore extracted in a Squibb's percolator with alcohol of 0.820 sp. gr., the whole being kept at 65° C. until 1500 cc. of extract were obtained.

The temperature was then raised to 75° and the extraction continued, about seven liters of alcohol being passed through the

meal. The last two liters were evaporated and left a residue weighing only 0.28 gram.

The meal residue was air-dried and extracted with ten per cent. sodium chloride solution. The extract was then filtered clear and saturated with ammonium sulphate, the precipitated proteid filtered out, dissolved in brine, the solution filtered perfectly clear and dialyzed.

The proteid was thus precipitated in large spheroids and was filtered out, washed with water and alcohol, dried over sulphuric acid and found to weigh 15.5 grams, preparation 13. This substance was freer from coloring matter than any before made, and had the following composition :

SUNFLOWER GLOBULIN, PREPARATION 13.

Carbon	51.54
Hydrogen	6.99
Nitrogen	18.58
Sulphur	1.00
Oxygen	21.71
	100.00
Ash	0.47

This preparation, which was very nearly white in color after drying, dissolved almost wholly in ten per cent. sodium chloride brine at 20°, giving a solution slightly tinged with greenish brown, which on dilution yielded an abundant precipitate that on warming, while suspended in the diluted solution, redissolved completely and again separated on cooling in spheroids, and on settling united with a coherent layer.

Solutions in ten per cent. sodium chloride brine behaved as follows :

When saturated with magnesium sulphate at 20° or sodium sulphate at 34°, the proteid was completely thrown out of the solution. When saturated with sodium chloride it was partly precipitated.

With mercuric chloride, picric acid, or tannic acid a heavy precipitate was produced.

With minute quantities of nitric, sulphuric, hydrochloric, or acetic acid the globulin was precipitated.

In pure water this preparation formed a plastic mass, but none dissolved.

In the water containing a minute quantity of acid it dissolved readily and completely.

With the xanthoproteic, Millon's, biuret and Adamkiewics' tests the usual preteid reactions were obtained.

When dissolved in ten per cent. sodium chloride solution and tested for heat coagulation point in the usual manner a turbidity formed at 90°, and a flocculent coagulum began to separate at 93°, increasing as the temperature was raised toward 100°. After heating sometime in a boiling water bath a considerable coagulum formed, yet a large proportion of the substance still remained in solution, as shown by the voluminous precipitate produced on adding acetic acid to the solution filtered from the coagulum.

In composition and reaction this preparation agrees with the globulin edestin, except that a part is precipitated by saturating its solutions in brine with sodium chloride. In composition the part precipitated by saturating with salt and that remaining in solution are alike. We have in another paper[1] pointed out that the globulin of the castor bean shows a similar behavior, and that the part precipitated by saturating with salt is a derivative of the part soluble in saturated salt solutions. We have further shown that the addition to a solution of edestin of a quantity of acetic acid too small to detect after mixing with the proteid, causes a precipitation of the edestin on saturating its solution with brine, and that under these conditions, the proteid otherwise behaves like the globulin from the castor bean and sunflower seed.

As helianthotannic acid contains about fifty-three per cent. of carbon, the presence of two per cent. of this acid in our preparation would but slightly raise the figures obtained for carbon and reduce those for nitrogen by about 0.35 per cent. The composition of the purer preparations which we have obtained differ from edestin to about this extent.

It is therefore our opinion that the sunflower seed contains as its principal proteid the globulin edestin, but that as obtained by extraction from the seed, this is mixed with helianthotannic acid, from which we have not succeeded in separating it completely.

Having thus found that a large part of this globulin is insolu-

[1] This Journal, 19, 482.

ble in saturated salt solutions under all the conditions of our tests, we were led to repeat Vines's experiments, but have been unable to confirm his observations, the aleurone grains appearing to be wholly unaffected by saturated salt solution after treatment of the seed with alcohol.

THE PROTEIDS OF THE COW PEA.[1]

(Vigna Catjang.)

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

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THE proteids of this plant have never been, to our knowledge, the subject of study. Because of its great and increasing agricultural importance, and as a plant differing botanically from those included in our investigation of "legumin," the proteids of its seeds have much interest. The material examined was prepared by coarsely grinding the peas, separating the black seedcoats by a current of air, and then grinding the coarse meal to a fine flour. Two kilograms of this flour were treated with a quantity of ten per cent. sodium chloride solution, the extract was strained through fine bolting-cloth and allowed for three hours to deposit the greater part of the suspended starch. The extract was then run through a DeLaval centrifugal separator, whereby most of the remaining suspended starch and fiber was removed, and lastly was filtered perfectly clear by passing through a thick layer of filter paper pulp. The extract was saturated with ammonium sulphate, the precipitated proteids collected on a filter and dissolved in brine. The solution was filtered perfectly clear and dialyzed for four days.

The proteid, thus separated in the form of spheroids, was designated *A*, and the solution filtered therefrom was marked *B*. *A* was collected on several paper filters. One portion was washed very thoroughly with water and with alcohol and, dried over sulphuric acid, gave preparation 1, which weighed 29.7 grams. The rest of *A* was dissolved in one liter of five per cent. sodium chloride brine, and the solution filtered perfectly clear. On adding one liter of distilled water a large precipitate, *D*, separated, which was allowed to settle over night. The liquid, *C*,

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