Carbon	51.40
Hydrogen	6.79
Nitrogen	16.43
Sulphur	1.03
Oxygen	24.35
	100.00

Owing to the insolubility of this substance in any but alkaline fluids and the difficulty of making preparations of known purity, nothing further was learned respecting it.

EFFECT OF MINUTE QUANTITIES OF ACID ON THE SOLUBILITY OF GLOBULIN IN SALT SOLUTIONS.[1]

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

Received April 12, 1897.

IN a paper on crystallized vegetable proteids by one of us[2] it is shown that the principal globulin of the seed of the castor bean is partly insoluble in a saturated solution of sodium chloride, and partly soluble therein, and that these two parts are alike in composition and but slightly different in reactions. Having found a proteid of similar composition and properties in the sunflower seed, we have again turned our attention to the globulin of the castor bean, with the hope that we might discover the cause of this partial precipitation by saturating its solutions with salt.

A considerable quantity of this globulin was prepared by extracting castor pomace with three per cent. brine at 60° and allowing the filtered extract to cool to the temperature of the room. The proteid thus separated was washed with water and alcohol, and dried over sulphuric acid. It formed a slightly colored dense powder consisting of a mixture of spheroids and octahedral crystals.

Seventy-five grams of this preparation were treated with 750 cc. of ten per cent. salt solution, and after agitating for some time, filtered from a large insoluble residue. This latter was washed thoroughly with ten per cent. brine and the filtrate and washings were united. In this way the substance was separated into two parts, one soluble and one insoluble, in cold salt solution. This solution was then saturated with sodium chloride

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

[2] Osborne: *Am. Chem. J.*, 14, 671.

and the large precipitate produced was filtered off, dissolved in 10 per cent. brine and this process twice repeated. The saturated sodium chloride solutions filtered from these precipitations were united and dialyzed free from salt; the proteid thus precipitated was washed with water and alcohol, and dried over sulphuric acid. Thus 12.39 grams of preparation A were obtained, representing the fraction of this globulin soluble in cold ten per cent. brine and *not* precipitated by saturation with sodium chloride. The proteid which had been several times precipitated from solution by saturation with salt, as just described, was dissolved in ten per cent. brine, and the solution filtered perfectly clear and dialyzed. By the usual treatment 18.52 grams of preparation B were obtained, representing the part of this globulin soluble in cold ten per cent. brine, but insoluble in saturated brine.

The part of the globulin which failed to dissolve in cold ten per cent. sodium chloride solution was next treated with salt solution of this strength, heated to 60° and allowed to cool to 14°. The greater part of the proteid was dissolved by this treatment, and after decanting the solution the undissolved residue was treated three successive times in the same way. The solutions obtained by this process were filtered clear from a slight quantity of suspended matters and saturated with sodium chloride, which precipitated all but an insignificant quantity of the dissolved proteid.

This precipitate was dissolved in ten per cent. brine, filtered clear and dialyzed. The precipitated globulin, after filtering off, washing, and drying, weighed 12.37 grams, and formed preparation C.

The part of the original globulin which failed to remain in solution after the above treatment with hot salt solution was dissolved in brine at 60°, filtered clear, and allowed to cool over night. Very nearly all the proteid precipitated on cooling, and was washed and dried, giving preparation D, weighing 1.66 grams.

These four preparations were analyzed with very great care with the following results :

	A.	B.	C.	D.
Carbon	50.99	51.10	51.12	51.25
Hydrogen	6.92	6.87	6.95	6.97
Nitrogen	18.95	18.67	18.83	18.74
Sulphur } Oxygen }	23.14	23.36	23.10	23.04
	100.00	100.00	100.00	100.00
Ash	0.19	0.14	0.14	0.41

The difference between these results barely exceed the usual errors of analysis, although several determinations of each element in the different fractions indicate that these differences are not due to analytical errors. It would not be safe, however, to take such slight variations into account, especially when we consider the great difficulty in making perfectly pure preparations of proteids as well as exact combustions. We must, therefore, conclude that no difference in composition is proved to exist between these four preparations which present such marked differences in solubility. A comparative examination of these substances was made with the following results:

In ten per cent. brine at 20°, A dissolved completely, B with the exception of a small residue, C partly, much being insoluble, while D did not dissolve at all. B and C dissolved nearly completely when warmed to 45°.

Five grams of each of A, B, and C were dissolved in 50 cc. of ten per cent. sodium chloride solution by heating to 50°. On cooling to 20°, B and C deposited a very slight amount of proteid, but on cooling to 12°, A gave a clear solution, while B deposited a not inconsiderable quantity of substance and C decidedly more. To five cc. of each of these solutions at 20° were added five cc. of water. A remained clear, B gave a slight precipitate, and from C practically all the dissolved globulin was thrown down, since further dilution of the solution filtered from this precipitate gave only a turbidity. With five cc. more water added to A a turbidity resulted, while the same amount added to B gave a heavy precipitate. The dilution of A and B was continued until the strength of the salt solution was 1.66 per cent., when B yielded no more by further dilution, and A still contained dissolved globulin.

Five per cent. solutions of each of these proteids, in ten per cent. brine, when heated became turbid. A and B at 88°, C at

87°, and flocculent coagula formed in A and C at 90° and in B at 91°, thus showing no difference in relation to heat.

Saturation with sodium chloride of the ten per cent. solution of this globulin gave a small precipitate in A, but completely precipitated B and C.

This partial precipitation of A shows that the substance precipitated by saturating with salt is a derivative of the body originally soluble in saturated brine.

In order to find the effect of minute quantities of acid added to these several fractions, two grams of each were treated with 20 cc. of 0.05 per cent. acetic acid, or five milligrams of acid for each gram of proteid, which caused no noticeable solution. Two grams of salt were added to each whereby A was largely, B partly, and C but slightly dissolved. Heated to 50°, A gave a clear solution, B a nearly clear solution, while C dissolved only partly and precipitated on cooling to 20°.

The acid added to these solutions could not be detected by very delicate litmus paper, the reaction being perfectly neutral.

A solution of A was prepared in exactly the same manner, omitting the acetic acid, and the two solutions compared.

Diluted with an equal volume of water, no precipitate formed in either solution, but with two volumes an abundant precipitate fell in that containing the acid, while only a very slight precipitate formed in the other. Saturated with sodium chloride, the solution with acid gave a large precipitate, that without acid only a small one.

It is thus clear that a quantity of acid too small to be detected with litmus or by analysis causes changes in the fractions soluble in saturated salt solution, whereby products result having the same general properties as those exhibited by the fractions B and C.

In order to obtain more evidence on this point, these experiments were repeated and extended, using crystallized edestin from hemp seed.

Five grams of edestin were suspended in 50 cc. of 0.05 per cent. acetic acid, five grams of salt were added, and the solution was warmed to 50° to dissolve the insoluble "albuminate" present.

Another solution was then prepared in exactly the same man-

ner without using acetic acid. Both solutions reacted perfectly neutral to litmus paper.

Equal volumes at the same temperature, and in test-tubes of the same size were immersed in the same bath of cold water. The solution containing acid precipitated first and in far greater amount than the other.

Five cc. of each were diluted with an equal volume of water at 20°. The solution with acid gave a much greater precipitate than the other. After allowing these to stand and cool to 10°, the precipitates were filtered off and one drop of strong acetic acid was added to each filtrate. The solution which had been made with acid gave only a turbidity, while a considerable precipitate formed in the other, showing that dilution precipitates the solution to which the acid had been added far more readily than the solution without the acid.

Equal volumes of these solutions were saturated with salt, the precipitates filtered off, and one drop of acetic acid was added to each filtrate. That from the solution made with acid gave only a turbidity, while the other gave a very heavy precipitate.

Here again we see that the addition of a quantity of acid, too small to detect after the solution has been made, brings about changes similar to those naturally occurring in the seeds and extracts of the castor bean and sunflower and to those following the addition of acid to that part of the globulin of the castor bean which is soluble in saturated salt solutions.

Whether such changes occur only through the influence of acids is a question not settled, and regarding which some doubt is raised by the fact that preparations of crystallized edestin which were originally soluble in ten per cent. sodium chloride solution with the exception of a small quantity of "albuminate" and yielded solutions which gave only traces of precipitates on saturating with sodium chloride, were found, after keeping dry and in cork-stoppered bottles two and four years, to have become largely insoluble in cold salt solution and to yield solutions which were nearly completely precipitated by saturating with salt. The insoluble portion dissolved nearly completely in ten per cent. brine at 60° to a solution precipitated somewhat by cooling to 20°, and abundantly at lower temperatures, copiously precipitated by dilution with an equal volume of water, and

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.