[FROM THE LABORATORY OF THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION.]

THE PRECIPITATION LIMITS WITH AMMONIUM SULPHATE OF SOME VEGETABLE PROTEINS.

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Received June 27, 1903.

HOFMEISTER and his students, who employed ammonium sulphate with much success in separating different proteins from one another, found that under suitable conditions the individual proteins are precipitated within quite narrow limits when this salt is added to their solutions up to certain degrees of saturation, which to a certain extent are characteristic for each protein. We have, therefore, applied this process to a number of the purer preparations of proteins which we had at our disposal.

A quantity of the protein was dissolved in one-tenth saturated ammonium sulphate solution, the solution filtered clear, and 2 cc. mixed with enough one-tenth saturated sulphate solution to make a final volume of 10 cc. with the saturated sulphate solution to be afterwards added. Successively greater quantities of the saturated solution were used and the points noted at which the solution first became permanently turbid, as well as that at which all the protein was precipitated, as shown by saturating the filtered solution with ammonium sulphate, and observing whether or not a precipitate or turbidity was produced. In many cases the filtrates contained minute quantities of something which was not precipitated with the bulk of the protein under examination, but could be afterwards precipitated from the filtrate by saturating with ammonium sulphate. In these cases we noted the point at which all but this trace was precipitated and also the point at which the solution finally became entirely clear. Whether this trace of substance, which is so much more difficult to precipitate than the bulk of the preparation, was a trace of contaminating proteose or some compound of the protein with some other substance or an alteration product produced by drying or in some other way during preparation, cannot now be told. However, in no case was more than an insignificant trace present and no serious contamination of the preparation is indicated by its presence. The "limits" here given are that at which the solution became turbid immediately after adding the saturated sulphate solution and that at which the protein

was first completely precipitated. Where a considerable further addition of sulphate was necessary to separate the final last trace, the point at which precipitation was practically complete is also given.

To determine the effect of concentration of the protein solution on the results of this determination, we tried the following experiments with neutral edestin.

Concentration of edestin solution. Per cent.	Lower limit. cc.	Upper limit. cc.
9.0	3.0	4.2
4.5	3.1	4.2
2.7	3.0	4.2

When 4.5 per cent. of edestin was dissolved in *10 per cent. sodium chloride*, the precipitation limits were lower, namely, 1.8 cc. and 3 cc. Edestin monochloride and edestin sulphate had essentially the same limits as the free edestin, although the lower limit for the sulphate was found to be a little below that of the others.

	Lower limit. cc.	Upper limit. cc.
2.37 per cent. of edestin monochloride.....	3.0	3.9
4.0 " " " " sulphate.....	2.5	4.2

The crystalline globulins of the squash-seed, flaxseed and castor bean are so nearly like that of the hemp-seed that until recently they have been regarded as the same protein. That this similarity extends to their relations toward ammonium sulphate is shown by the following figures:

	Protein. Per cent.	Lower limit. cc.	Upper limit. cc.
Edestin hemp-seed.....	2.7	3.0	4.2
Globulin flaxseed.....	3.4	3.1	4.7
Globulin squash-seed	3.6	3.3	4.4
Globulin castor bean.....	..	3.1	4.5

The globulin of the cottonseed, which we now recognize as another protein than edestin from the hemp-seed, has different precipitation limits from edestin, namely, for a solution containing 2.5 per cent. of globulin, a lower limit of 4.6 cc., and an upper one of 6.4 cc. In this we haye another distinction between the cotton-seed globulin and edestin. The globulin of the filbert (*Corylus tubulosa*) and that of the English walnut (*Juglans regia*) were

described by Osborne and Campbell[1] under the name of corylin, since a careful comparison of the reactions and analysis of a number of different preparations showed no difference between them. We have recently found[2] that the globulin of the filbert yields a little more ammonia, when decomposed with hydrochloric acid, than does the globulin of the English walnut, and we must therefore consider them to be distinctly different substances. This conclusion is supported by our determinations of the precipitation limits.

A solution containing 3.4 per cent. of the globulin from the filbert became turbid with 3.7 cc., was almost wholly precipitated with 5.3 cc., and completely with 6.6 cc. Under the same conditions a solution containing 2.75 per cent. of globulin from the English walnut became turbid with 2.8 cc., was almost wholly precipitated by 4.6 cc. and completely precipitated with 6.6 cc. That the last traces were precipitated, after nearly all of the globulin had been thrown out of solution, only by adding a considerably greater quantity of sulphate, indicates a slight contamination of the preparation with some other protein, possibly a trace of adhering proteose, possibly an alteration product of the globulin formed during drying or otherwise. However this may be, the amount was too small to be of serious consequence. The globulin of the American black walnut showed exactly the same behavior toward ammonium sulphate as that of the English walnut.

It is hence evident that the globulin of the English walnut is a different substance from that of the filbert and the name corylin should therefore be applied only to the latter.

Excelsin from the brazil nut and amandin from the almond have nearly the same precipitation limits although, otherwise, they are distinctly different substances. The lower limit for excelsin was found to be 3.8 cc., the upper 5.5 cc., while for amandin the lower limit was 3.5 cc. and the upper limit 5.3 cc. Preparations of legumin from different seeds, which have as yet appeared to be in all respects alike, showed the same precipitation limits with ammonium sulphate.

	Lower limit. cc.	Upper limit. cc.
1.8 per cent. legumin, vetch.....	5.2	7.3
3.2 per cent. legumin, horse bean.....	5.4	7.5
2.6 per cent. legumin, lentil.....	5.5	7.4

[1] This Journal, 18, 609 (1896); also Report of the Conn. Agr. Expt. Station for 1895, p. 288.

[2] This Journal, **25**, 423 (1903).

Nearly all the legumin was precipitated in each case by 6.5 cc., that remaining in solution with more than this quantity being very little.

The globulin of the castor bean is partly precipitated from its concentrated solutions in 10 per cent. sodium chloride by saturating its solution with this salt. The precipitate thus produced, when redissolved in dilute brine, is again partly thrown out by saturating with sodium chloride, and as often as this process is repeated a considerable part of the globulin remains dissolved in the saturated salt solution. From this it seems more probable that the globulin has a limited solubility in saturated sodium chloride solution, which is much less than that in a 10 per cent. brine, than that two different globulins exist in the seed. The precipitation limits with ammonium sulphate also indicate that this is the case, as the following experiment shows. A quantity of this globulin, obtained by dialyzing a sodium chloride extract of castor beans, was dissolved in a moderate quantity of 10 per cent. brine, and the solution saturated with sodium chloride. The large precipitate was filtered out, again dissolved in brine, and the solution saturated with sodium chloride. The second precipitate appeared to be less than the first. The solution and precipitate were again repeated, when the final precipitate was dissolved in brine, and this solution, as well as the three filtrates from the precipitates produced by saturation, were dialyzed till free from sodium chloride. The substance separated from all four solutions as a mixture of crystals and spheroids, which were washed with water and dehydrated with absolute alcohol. After drying over sulphuric acid, the different preparations weighed as follows:

No. 1. 8.67 grams. This was the final precipitate produced by saturating with sodium chloride.

No. 2. 6.2 grams. The globulin remaining in solution after precipitating No. 1.

No. 3. 13 grams. The globulin remaining in solution after the second precipitation.

No. 4. 12.8 grams. The globulin remaining in solution after the first precipitation.

Since the substance yielding 1, 2 and 3 was precipitated by the first saturation with sodium chloride and two-thirds of this remained in solution after saturating the second and third time, it is

evident that precipitation is simply due to a limited solubility of this globulin in saturated salt solution. The smaller weight of No. 2 is due to the smaller volume of the solution from which it was obtained.

The precipitation limits of these preparations were as folows:

	Lower limit. cc.	Upper limit. cc.
No. 1	3.1	4.4
No. 3	3.16	4.5
No. 4	3.4	4.8

The slightly higher limits shown by No. 4 differ too little from those of the others to warrant the conclusion that two different proteins exist in these preparations.

The seeds of the yellow lupine contain a large amount of protein matter which can be separated into two extremes by fractional precipitation, which differ slightly from one another in properties and composition, especially in sulphur content, the more soluble fraction containing three times as much sulphur as the less soluble.

The precipitation limits were as follows:

	Lower limit. cc.	Most precipitated between. cc.	cc.	Upper limit. cc.
Conglutin (*a*) less soluble	4.2	4.3	6.0	7.3
Conglutin (*b*) more soluble	4.6	6.4	8.2	8.7

From these results it is evident that the two extremes represent different proteins and that the more soluble one is precipitated at a much higher saturation than the less soluble one. It also appears that the separation of these two proteins from one another in the case of the preparations here tested, was not entirely complete.

The globulin from the blue lupine showed nearly the same behavior as the less soluble globulin of the yellow lupine, the lower limit being 4.4 cc., while with 6 cc. nearly all was precipitated.

Phaseolin is more soluble in strong solutions of ammonium sulphate than any protein we have yet examined. The lower limit was found with 6.4 cc. With 8.2 cc., which was all that could be added, under the conditions of the test as applied to the other globulins, there was still considerable protein in solution. When 1 cc. of the phaseolin solution was mixed with 9 cc. of the saturated solution all was precipitated. The upper limit appears to be, therefore, a little below 9 cc. The following table contains the

results of these determinations, arranged in the order of the solubility of each protein substance in ammonium sulphate solution.

Protein.	Lower limit. cc.	Most precipitated. cc.	cc.	Upper limit. cc.
Globulin, English walnut	2.8	2.8	4.6	6.6
Globulin, black walnut	2.8	2.8	4.6	6.6
Edestin	3.0	3.0	4.0	4.2
Edestin monochloride	3.0	3.0	3.9	3.9
Globulin flaxseed	3.1	3.3	4.6	4.7
Globulin castor bean	3.1	3.3	4.3	4.5
Globulin squash-seed	3.3	3.5	4.1	4.4
Amandin	3.5	3.5	5.0	5.3
Corylin	3.7	3.7	5.3	6.6
Excelsin	3.8	4.0	5.0	5.5
Conglutin (*a*)	4.2	4.3	6.0	7.3
Conglutin (*b*)	4.6	6.4	8.2	8.7
Globulin cottonseed	4.6	5.0	6.0	6.4
Legumin	5.4	5.5	6.5	7.5
Phaseolin	6.4	6.5	8.2	8.8

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THE SPECIFIC ROTATION OF SOME VEGETABLE PROTEINS.

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Received June 27, 1903.

THE specific rotation of very few of the vegetable proteins has been determined, the only observations, so far as we know, being those of Kjeldahl[1] on zein and gliadin, Alexander[2] on edestin, excelsin and the globulin of the flaxseed, and Chittenden and Mendel[3] on edestin. Having had an opportunity to make some observations on the proteins above named, as well as on a few others, we take this occasion to put then on record.

The determinations were made with a Schmidt and Haensch half-shade polariscope, provided with a sugar scale. The readings were calculated to degrees of circular polarization by multiplying the degrees observed on the sugar scale by 0.346. The results

[1] Kjeldahl: *Agricultur. Chem. Centrbl.*, **25**, 197 (1896).
[2] Alexander: *Jour. Expt. Med.*, **1**, No. 2 (1896).
[3] Chittenden and Mendel: *Jour. Physiol*, **17**, 40 (1894).