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THE PROTEINS OF THE PEA (*Pisum Sativum*).[1]

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According to former investigations made in this laboratory the seeds of the garden pea (*Pisum Sativum*) contain three different proteins: Legumin, vicilin and legumelin. The two former are globulins of similar composition and properties which were separated from one another by fractional precipitation from sodium chloride solutions, the vicilin being more soluble than the legumin in dilute saline solutions while the legumelin remained in solution after dialysis and was separated by heating the solution to 80°. The most marked difference between legumin and vicilin was shown by the behavior of their solutions on heating. Sodium chloride solutions of legumin remain perfectly clear when heated to 100°, those of vicilin become turbid at 90°, at 95° a flocculent coagulum separates and when heated at 100° for some time the vicilin is almost completely coagulated. Legumin contains a little less carbon and a little more nitrogen than vicilin and distinctly more sulphur. Vicilin contains less sulphur than any protein thus far isolated. By repeated precipitation the amount of sulphur was found to diminish from 0.23 per cent to 0.08 per cent.[2]

The name legumin has long been used to designate various protein preparations obtained from many of the leguminous seeds. Most of these preparations were formerly obtained by extraction with alkali and precipitation with dilute acid and therefore represented products of doubtful character. Later investigations showed that most of these proteins were globulins which could be

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] Cf. Osborne and Campbell: *Journ. Amer. Chem. Soc.*, xx, p. 410, 1898.

extracted by sodium chloride solution and that several of the preparations previously called legumin were certainly different substances. The investigations of many different leguminous seeds made in this laboratory have shown that from the pea, *Pisum Sativum*, horse bean, *Vicia Faba*, lentil, *Ervum lens*, and vetch, *Vicia Sativa*, preparations of the globulin can be obtained which agree strictly in properties and composition with one another but are distinctly different from those obtained from seeds of the genus *Phaseolus* and other kinds of legumes. The writer has therefore designated this protein as legumin, for it undoubtedly represents the substance to which earlier investigators most frequently intended to apply this name. In the seeds of the pea, horse bean and lentil the legumin is associated with another protein which has been called vicilin by the writer. The history of legumin and the characteristics of the proteins of the seeds above mentioned has been discussed in papers from this laboratory.[1]

Legumelin is an albumin-like protein which is not precipitated by dialysis and is coagulated by heating its solutions to about 80°. The greater part of the legumelin separates between 60° and 65° but its complete coagulation is not effected below 80°.

The composition of legumelin is distinctly different from that of legumin or vicilin. In properties and composition it closely resembles leucosin found in the embryo of wheat and it is probably a tissue protein rather than a reserve food protein of the endosperm. Proteins of the same composition and properties as the legumelin of the pea have been found in a large number of other leguminous seeds, *e.g.*, lentil, horse bean, vetch, adzuki bean, cow pea and soy bean.

As a complete separation of legumin from vicilin by fractional precipitation from sodium chloride solutions can be obtained only by the sacrifice of a large part of the mixed globulins and the expenditure of much time and labor we have studied the results obtained by fractional precipitation with ammonium sulphate and found that this method yields products of the same properties and ultimate composition as those previously obtained from

[1] Cf. Osborne and Campbell: *Journ. Amer. Chem. Soc.*, xviii, p. 583, 1896. *Ibid.*, xx, pp. 348, 362, 393, 406 and 410, 1898.

sodium chloride solutions. By this method it is possible to prepare large quantities of these proteins without much loss of material and with comparative ease.

The results given by this method are shown by the following experiment:

The pea-meal was extracted with 10 per cent sodium chloride solution and the extract, filtered clear, was saturated with ammonium sulphate. The precipitate thus produced was dissolved in dilute ammonium sulphate solution and the resulting solution dialyzed for five days. The precipitated globulin was dissolved in sodium chloride solution and again precipitated by saturation with ammonium sulphate, the precipitate dissolved in sodium chloride solution and, after filtering perfectly clear, the solution was dialyzed for ten days. The dialysis precipitate was then suspended in 1000 cc. of water and dissolved by adding 76 grams of ammonium sulphate. By adding 380 grams more ammonium sulphate, thereby raising the concentration to six-tenths saturation, a considerable precipitate was produced. This was filtered out, washed with six-tenths saturated sulphate solution, dissolved in dilute sulphate solution and the resulting clear solution dialyzed for seven days. The globulin that precipitated was completely soluble in 10 per cent sodium chloride solution and gave no coagulum when this solution was boiled.

The remainder of the globulin, when washed with water and alcohol and air-dried, weighed 15.3 grams and, dried at 110°, had the following composition: C, 51.74; H, 7.14; N, 17.77 per cent.

The filtrate from the precipitate produced by six-tenths saturation was raised to seven-tenths saturation but, as only a trace of precipitate formed, the saturation was raised to eight-tenths and the resulting precipitate filtered out. This was dissolved in dilute ammonium sulphate solution and the clear solution was dialyzed for seven days. The precipitate that formed, when filtered out, washed and air-dried, weighed 7.35 grams. It was completely soluble in dilute sodium chloride solution and largely coagulated when this solution was heated in a boiling water-bath.

Dried at 110° this preparation had the following composition: C, 52.25; H, 7.28; N, 17.17 per cent. The solution filtered from the precipitate at eight-tenths saturation, when completely saturated with ammonium sulphate, yielded a small precipitate which

when redissolved and precipitated by dialysis, gave 0.69 gram of substance which was wholly soluble in dilute sodium chloride solution, coagulated by heating to 100° and when dried at 110° had the following composition: C, 52.17; H, lost; N, 17.08 per cent. A repetition of this experiment, making the separation at seven-tenths saturation, gave essentially the same results, namely, 26.5 grams of legumin, containing C, 51.89; H, 6.83: N, 17.78 per cent, and 9.5 grams of vicilin, containing C, 52.35; H, 7.15; N, 16.90 per cent.

A third extraction gave at five-tenths saturation 17.76 grams of globulin containing 17.77 per cent of nitrogen; between five-tenths and six-tenths 9.62 grams containing 17.99 per cent of nitrogen; between seven-tenths and eight-tenths 10.03 grams containing 17.18 per cent nitrogen, and between eight-tenths and complete saturation 7.46 grams, containing 17.00 per cent of nitrogen.

It is thus evident that separation by fractional precipitation with ammonium sulphate yields products of the same composition and properties as those formerly obtained by fractional precipitation from sodium chloride solutions and as this separation is effected without difficulty and with little loss of substance, it affords an excellent method for preparing large quantities of these two proteins in as pure a state as it is possible to obtain them in any way known to us.

We accordingly prepared large quantities of these proteins for hydrolysis by extracting the pea-meal with 10 per cent sodium chloride solution, filtering the extract perfectly clear and dialyzing until nearly all of the chlorides were removed. The dialysis precipitate was then dissolved in one-tenth saturated ammonium sulphate solution, the resulting solution filtered clear, and enough ammonium sulphate crystals dissolved in it to bring it to six-tenths saturation.

The precipitate thus produced was dissolved in sodium chloride solution and, after filtering perfectly clear, the solution was dialyzed until free from chloride. The resulting precipitate of legumin was then washed with water, dilute and absolute alcohol and dried over sulphuric acid.

The filtrate from the precipitate produced by six-tenths saturation with ammonium sulphate was completely saturated with

this salt, the precipitated protein dissolved in sodium chloride solution and, after filtering perfectly clear, the solution was dialyzed until free from chlorides. After washing the dialysis precipitate with water and alcohol it was dried over sulphuric acid. The product thus obtained formed our preparation of vicilin. The filtrate from the precipitate produced by the first dialysis of the original sodium chloride extract of the pea-meal was heated to 80° in a water-bath, the voluminous coagulum was washed with water and dehydrated with absolute alcohol, giving a preparation of legumelin. By this method 1840 grams of legumin, 865 grams of vicilin and 790 grams of legumelin were obtained.

This legumin contained 17.75 per cent of nitrogen (Kjeldahl), 0.46 per cent of sulphur and 0.48 per cent of ash, and when dissolved in sodium chloride solution was not coagulated by heating to 100°. The vicilin contained 17.15 per cent of nitrogen, 0.26 per cent of sulphur and 0.41 per cent of ash. Dissolved in sodium chloride solution it was abundantly coagulated on heating to 100°.

These preparations of legumin and vicilin agreed in composition and deportment on heating with those obtained by fractional precipitation from sodium chloride solution and undoubtedly represent the same fractions of the total globulin of this seed as were formerly described under these names.