Seed Globulins of the Gramineae and Leguminosae

By C. E. DANIELSSON, *Institute of Physical Chemistry, Uppsala*

(Received 17 June 1948)

1. SEED GLOBULINS OF THE MOST COMMON SPECIES OF THE GRAMINEAE AND THEIR DIFFERENTIATION IN THE SEED

The globulins in the seeds of different species of grasses do not occur in large amounts. The following values, taken from Osborne (1909), refer to seeds of wheat: glutenin, 4.68; gliadin, 3.96; globulin, 0.62; albumin, 0.39; 'proteose', 0.21 %. It is evident that the globulins comprise only about 6 % of the total protein. This naturally causes difficulties in carrying out investigations since yields are small.

A very valuable contribution to the knowledge of the vegetable proteins was made by Quensel (1942) in his investigations of the seed globulins of barley. His method for isolating them was based on Osborne's (1909) earlier work, and made use of the solubility of globulins in dilute salt solutions, and their precipitation by ammonium sulphate or by dialysis against water.

EXPERIMENTAL AND RESULTS

General method for the preparation of globulin fractions

Since Quensel's (1942) method of preparation was followed to a large extent in these investigations, only a brief account will be given here. Quensel extracted overnight at 4° c. 800 g. of barley meal with m-NaCl, buffered with phosphate to pH 7.0. By fractional precipitation of the extract with solid $(\text{NH}_4)_2\text{SO}_4$, after clarifying by filtration, three fractions were obtained. By dissolving the precipitates in 0.2M-NaCl, pH 7.0, and subsequent dialysis of the solution in a cellophan sac against distilled water, the globulins were separated from the albumins, low-molecular and polydisperse material. In this way there could be obtained from barley seeds three fractions containing varying amounts of four well defined globulins called respectively α , β , γ and δ , in order of increasing molecular weight. These components were distributed amongst the different fractions in the following way: (1) 15% saturation with $(\text{NH}_4)_2\text{SO}_4$, β -rich fraction; (2) 40% saturation with $(\text{NH}_4)_2\text{SO}_4$, polydisperse fraction; (3) 70% saturation with $(\text{NH}_4)_2\text{SO}_4$, α -, γ - and δ -rich fraction.

It may be noted here that the δ -component, as shown by later investigations, only appeared occasionally in the sedimentation diagrams, and then only in small concentrations.

Method of investigation

This for the most part was based on ultracentrifugation (see Svedberg & Pedersen, 1940). In the presentation of sedimentation constants, Svedberg units have been used

($1 S = 10^{-13} \text{ cm. sec.}^{-1} \text{ dyne}^{-1}$). In the diagrams, the distance from the centre of rotation is plotted on the abscissa and the displacement of the scale divisions (Z) on the ordinate. To the left is the meniscus of the liquid at a distance of 5.8–6.0 cm. from the centre of the rotor, and to the right is the bottom of the cell, about 7.2 cm. from the centre. Since the displacement of the scale divisions is proportional to the concentration gradient in the cell, the area between the curve and the abscissa is proportional to the concentration. Each peak in the diagram corresponds to a protein component.

Fig. 1. Sedimentation diagrams of barley globulins. Upper: fraction obtained at 15% saturation with $(\text{NH}_4)_2\text{SO}_4$. Lower: 70% saturation. In all the sedimentation diagrams in this paper the abscissae represent distance from the centre of rotation (cm.), the ordinates represent the scale-line displacement (μ .), and sc = distance from scale (mm.). Time of sedimentation is given in min.

Quensel (1942) found the following values of the sedimentation constants for the globulin components isolated from barley: $\alpha = 2.49 S$, $\beta = 6.21 S$, $\gamma = 8.30 S$, $\delta = 12.0 S$. Although he did not succeed in isolating any of these components separately, he worked out a method for determining the molecular weights of different components in

* 100% saturation = 760 g. $(\text{NH}_4)_2\text{SO}_4/\text{l.}$

mixtures of this kind. The molecular weight is calculated from the Svedberg formula $M = \frac{RTs}{D(1 - V\rho)}$, where R = the gas constant; T = absolute temperature; V = partial specific volume; ρ = density of the solvent; M = molecular weight; s = sedimentation constant and D = diffusion constant. Taking $V = 0.72$ and the above values for s_{20} together with the values of the diffusion constants determined by Quensel's special method ($D_\alpha = 8.4$ and $D_\gamma = 4.4$ in 10^{-7} c.g.s. units), $M_\alpha = 26,000$ and $M_\gamma = 166,000$. The molecular weights for β and δ were estimated from their sedimentation constants to be $M_\beta = 100,000$ and $M_\delta = 300,000$. Typical sedimentation diagrams from barley are given in Fig. 1. The α and γ components always appear at the same time in the sedimentation diagrams, as mentioned above. Quensel tried to separate these two components, but this proved impossible with his methods, which included electrophoresis and centrifugation in an air centrifuge. He points out that no genetical relation between the components could be established, and further that there was no possibility of deciding on the basis of his investigations whether or not the different components were differentiated in the seed itself. The last question has been taken for investigation in this work.

The globulins of Gramineae other than barley

Since Quensel's work only applied to barley it is of interest to see in what degree these globulin components are found in other species of the same plant family. In investigations of this point the preparation scheme of Quensel has been followed, and the results which were obtained are given in Table 1 and

Table 1. *Globulins of species of Gramineae*

Species	Components				Sedimentation constants (S)			
	α	β	γ	δ	2.5	6.2	8.3	12.0
<i>Hordeum vulgare</i>	α	β	γ	δ	2.5	6.2	8.3	12.0
<i>Secale cereale</i>	α	—	γ	—	2.6	—	8.2	—
<i>Triticum vulgare</i>	α	—	γ	—	2.5	—	8.2	—
<i>Avena sativa</i>	α	—	γ	—	2.6	—	8.1	—
<i>Zea Mays</i>	α	—	γ	—	2.6	—	8.5	—
<i>Panicum miliaceum</i>	—	—	γ	—	—	—	8.5	—
<i>Phleum pratense</i>	—	—	γ	—	—	—	8.2	—
<i>Festuca pratensis</i>	—	—	γ	—	—	—	8.3	—
<i>Festuca rubra</i>	α	—	—	—	2.4	—	—	—

Fig. 2. The investigation had to be limited to the species occurring most commonly in commerce. It appears that the protein content varies greatly with the different species, and with some species is remarkably low. This is particularly true of *Phleum pratense* and the *Festuca* species, the seeds of which are very small, and seem to contain very small amounts of reserve protein. The preparation of globulins from these species is thus difficult and a negative result must not be considered as an absolute sign of their absence. Earlier work (Säverborn, Danielsson & Svedberg, 1944) has shown that malt contains the same components as barley, and the sedimentation diagrams from barley and malt are identical.

Quensel's investigations showed that the sedimentation constant of the γ component depends on concentration, i.e. $s = 8.30 - 0.6c$, where c is the percentage concentration and 8.30 is the sedimentation constant at infinite dilution ($c = 0$). The values in Table 1 have been corrected with the help of this equation. The concentrations have been obtained partly by measurement of the refractive index of the solution, and partly by calculation from the sedimentation diagram.

Hordeum vulgare (barley) is unique in this family, since it alone contains the β component, which occurs in high concentration. Barley is also the only species which contains the δ component, although only in low concentration.

The β component has some very interesting properties, which may possibly be connected with certain phenomena in the brewing of beer. The relations are not altogether clear, but it seems possible that the β component is part of the precipitate which is obtained on storing beer in the cold. Investigations at this Institute show that in this precipitate the β component may be combined with tannic acid. The β component is very stable towards many agents such as heat and enzymes, and it has been possible to show (Säverborn *et al.* 1944) that it survives the mashing process in the production of beer which causes degradation of the other proteins. The β component is distinguished from the other components by its content of 1.96 % S, compared with 1.65 % for the α and γ components. These proteins thus differ not only in molecular weight but also in chemical constitution. This partly explains the large differences on salting out with ammonium sulphate.

If these extra components in barley are disregarded, the different species within the family seem to have, to a large extent, the same seed globulins. It is to be noticed that the γ component is predominant in the species that have little reserve protein, for example, *Phleum pratense* and *Festuca pratensis*. This is understandable in the light of our experiments, when it was found to occur only in the embryo: a very small seed with little reserve protein would thus be expected to contain mainly the γ component.

Differentiation of the components in the seed

Experiments with wheat. The question of the differentiation of the globulin components defined by Quensel (1942) was partly solved by the work on wheat (Säverborn *et al.* 1944). The separation of the components was made possible by the fact that, in the milling of wheat, one can separate the embryo from the rest of the seed.

In the commercial milling of wheat a number of different fractions is obtained. Only the most interesting fractions have been investigated here. The

Fig. 2. Sedimentation diagrams showing the composition of globulin solutions prepared by extraction of seeds from different species of the Gramineae. The four figures to the left refer to different fractions of seeds from wheat. Ordinates and abscissae as in top left.

results obtained earlier from investigations on the whole grain were compared with those obtained by the same method on the following fractions: (a) bran fraction; (b) embryo fraction; (c) flour fraction with husk and embryo removed.

The sedimentation diagrams of the globulins obtained from the various fractions were practically identical with those from barley, with the important exception that the β component was absent. Exactly the same sedimentation constants were obtained. Only in respect of the concentrations of the α and γ components could a difference be observed, as can be seen from Fig. 2. The γ component is obtained completely pure from the embryo fraction, while the flour fraction contains considerably more α than γ . Finally, the bran fraction contains γ in much higher concentrations than α . This fraction contains the so-called aleurone layer, which is particularly rich in proteins.

Experiments with barley. In barley, the embryo cannot be separated directly by milling, but was obtained manually by excision with a razor blade. The two fractions (embryo and residual fraction) were investigated by the same method as before, using 100 g. of embryo and 200 g. of residual fraction for each experiment. The result obtained was in complete agreement with the above-mentioned experiments with wheat seed. The sedimentation constants were naturally identical for both barley and wheat, and equal to those obtained by Quensel (1942) for solutions containing both components.

For barley, the α component occurs entirely in the residual fraction except for a low concentration in the embryo where it is present probably as a contaminant, being introduced along with the residual fraction which adheres to the embryo when the latter is removed from the seed manually. This finding is in accord with the observation that with wheat, as a result of the nearly perfect isolation of the embryo by the milling process, the α component is found only in the residual fraction.

The γ component occurs only in the embryo fraction of both wheat and barley, being isolated completely pure from wheat and only slightly contaminated with α , as mentioned above, in the barley preparation. Finally, the β component has been found only in barley seeds where it is present solely in the residual fraction.

The distribution of the different globulin components in the seeds of wheat and barley is shown in Table 2 and Fig. 3. It is of interest that the protein of highest molecular weight is in the embryo, the most vital part of the seed. It is perhaps premature to draw conclusions concerning the utilization of the proteins in the germination itself and in the formation of the young plant, but it is conceivable that the different components have different functions in germination. The latter causes a large diminution in

the concentrations of these components, and it is reasonable to suppose that they are directly broken down to smaller units, which can be easily utilized by the young plant.

Table 2. *Distribution of globulins within the wheat and barley seed*

	Embryo fraction			Residual fraction		
	α	β	γ	α	β	γ
Wheat	-	-	+++	+++	-	+
Barley	+	-	+++	+++	+++	-

Fig. 3. Diagram showing the localization of the α and the γ components in seeds of wheat. *Al*, aleurone grains; *E*, embryo.

Whether there exists a biogenetical relationship between the globulin components described here is very difficult to decide. Many attempts have been made to degrade in different ways the purified γ component from wheat embryo in order to find out whether the α component can be obtained as an intermediate product, but without success. Ultrasonic vibration had no influence on the γ component, whilst among the proteolytic enzymes, papain

caused a very slow but continuous degradation under optimal conditions, and the process could easily be followed in the ultracentrifuge. No well-defined high-molecular intermediate products have been found under any circumstances, leaving the question of a possible relationship between the components unsettled.

(1937). Fig. 4 shows the results of the diffusion measurements for the pure γ component from wheat embryo. The diffusion constant has been calculated by two methods, which give the values D_m and D_A . For a substance having ideal diffusion both methods must of course give the same value for D . The values in Table 3 have been calculated from the curves in

Fig. 4. Diffusion diagrams of the γ component of wheat embryo. *A*, the curves marked I, II and III refer to exposures taken 29, 49 and 73 hr. after the beginning of the diffusion process and show the concentration gradient as a function of the distance from the originally sharp boundary between solvent and solution (0). *B*, the diffusion curves I, II and III from *A* have been transformed to normal co-ordinates, marked by small circles. The larger circles and the curve correspond to an ideal diffusion curve. Z =displacement of scale divisions.

Determinations of the molecular weights of the α and γ components

The methods described above for the isolation of the α and γ components have been used for the preparation of pure components for diffusion measurements. In this way α has been prepared from barley after removal of the embryo, while the γ component has been obtained from the wheat embryo.

In the determination of the molecular weight of the γ component, the concentration dependence of the sedimentation constant must be taken into consideration. In this investigation the value $s_{20} = 8.7 S$ at infinite dilution was obtained for the γ component of wheat embryo, whereas Quensel (1942) found the value $8.30 S$, or about 5% less for γ from barley. However, in Quensel's experiments, the sedimentation of the γ component was disturbed by the presence of α , the contribution of which to the viscosity of the medium was not known and was accordingly omitted from the calculations. The sedimentation constant reported here for wheat γ is presumably correct since the preparation was pure. The apparent difference, therefore, in s_{20} for γ globulin from wheat and barley may be merely a result of experimental inaccuracy.

The diffusion measurements have been made with the help of the scale method worked out by Lamm

Fig. 4. There is a steady decrease with time in the values for both D_m and D_A , which has persisted in later experiments. $D = 3.6 \times 10^{-7}$ c.g.s. is taken as the average.

Table 3. D_{20} of the γ component of wheat embryo

(D is given in units of 10^{-7} c.g.s. at 20° .)

	Diffusion time (hr.)					Average
	29	36	49	57	73	
D_m	3.72	3.71	3.52	3.41	3.44	3.56
D_A	3.76	3.71	3.57	3.58	3.46	3.62

Table 4. D_{20} of the α component of barley

(D is given in units of 10^{-7} c.g.s. at 20° .)

	Diffusion time (hr.)					Average
	9	22	35	45	59	
D_m	7.99	7.64	7.64	6.81	7.08	7.43
D_A	7.78	7.35	7.34	6.79	7.10	7.27

From the values $s_{20} = 8.7 S$, $D = 3.6 \times 10^{-7}$ c.g.s., $V = 0.72$ (Quensel, 1942) and $\rho = 0.9982$, the molecular weight $M_\gamma = 210,000$ is obtained. This exceeds

Quensel's value by 28 %, the difference being due to the fact that we used the isolated and completely pure γ component, which led to a greater accuracy of measurement. The values of D for the α component of barley are given in Table 4. The agreement

between D_m and D_A may be considered satisfactory. By using $s_{20} = 2.5 S$, $D = 7.4 \times 10^{-7}$ c.g.s., $V = 0.72$ (Quensel) and $\rho = 0.9982$ one gets $M_\alpha = 29,000$, a value which thus lies very near to that found by Quensel.

2. SEED GLOBULINS OF THE LEGUMINOSAE

The seeds of leguminous plants are very rich in proteins, containing 30–50 % total protein compared with 7–10 % for members of the Gramineae. It was assumed, therefore, that these globulins would be easier to prepare than those of the Gramineae, and this proved to be so.

Careful investigations of globulins from peas have been carried out by Osborne (1898). Apart from elementary analyses, Osborne studied chiefly the solubility and coagulation properties of the globulins. From these latter studies Osborne was able to isolate three globulin fractions from peas: (1) legumin, does not coagulate on heating to 100° ; (2) vicilin, coagulates on heating to 95 – 100° , soluble in more dilute salt solutions than legumin; (3) legumelin, occupies an intermediate position with respect to globulins and albumins and is partly precipitated on dialysis.

In the experiments which will be described, Osborne's method of preparation has mainly been used, viz. precipitation with ammonium sulphate and dialysis. Our preparations, therefore, should be very similar to those which Osborne analyzed. The same scheme of preparation has been employed here as in the earlier experiments on the Gramineae; seeds of *Pisum sativum* have been mainly used.

Experimental and results

The sodium chloride extract of the ground seeds was at first precipitated with solid ammonium sulphate at three different degrees of saturation, 15, 40 and 70 %. Ultracentrifuge diagrams of the three fractions are all similar, and indicate two components (Fig. 5), with sedimentation constants about 7.5 and 12.5 S . Since the different fractions contained the same globulin components in about the same concentration the extract was subsequently precipitated directly at 70 % saturation. The following scheme of preparation has been used.

Finely ground seeds are extracted for 1 night at 4° with 10 times their weight of M -NaCl, buffered with phosphate to pH 7. The solution is then centrifuged, and the extract filtered clear. Solid $(NH_4)_2SO_4$ is added with stirring to 70 % saturation (532 g. of salt/l.) and the precipitate centrifuged down. It is dissolved in 0.2 M -NaCl, buffered to pH 7. After filtration, the solution obtained is dialyzed against running water in a cellophan sac. After 12–24 hr. the globulins have precipitated. They are recovered by centrifuging, the precipitate is redissolved, and the solution dialyzed once more until precipitation has occurred. The precipitate obtained from the second dialysis is dried *in*

vacuo over $CaSO_4$. In this way, a yellowish white powder is obtained which is very readily soluble in salt solutions at pH 7. A buffer with the following composition has been

Fig. 5. Sedimentation diagrams showing the globulin composition in seeds of *Pisum sativum*: upper, 15 % saturation with $(NH_4)_2SO_4$; centre, 40 %; lower, 70 %.

used here: 0.2 M -NaCl; 0.03 M - Na_2HPO_4 ; 0.02 M - NaH_2PO_4 ; pH 7.0. This buffer will be referred to as the standard buffer. The dried preparation can be stored indefinitely, and the solubility does not change with time (no change has been noticed during 1 year).

The yield varies of course from preparation to preparation, depending on how completely the substance precipitates during the two dialyses. Usually a yield of 1.5–2.0% of the pure components is obtained. Peas contain 21.5% total protein (Czapek, 1905). The globulins, therefore, comprise about 10% of the total protein.

mentation diagram of the 70% saturation fraction from peas.

In order to obtain more reliable values of the sedimentation constants, the centrifugations have been carried out at different concentrations. The results are given in Fig. 7. Thus, for the component with the higher sedimentation constant, no de-

Fig. 6.

Fig. 6. Sedimentation diagrams of the fraction obtained at 70% saturation with $(\text{NH}_4)_2\text{SO}_4$ (*Pisum sativum*). The diagrams correspond to exposures taken 20, 35 and 65 min. after the beginning of sedimentation at 65,000 r.p.m.

Fig. 7.

Fig. 7. The relation between the sedimentation constants and the concentrations for the two globulin components in seeds from pea.

Investigation of pea globulins in the ultracentrifuge

As shown earlier (Fig. 5), two components are obtained on ultracentrifuging the globulin fractions prepared as above. Fig. 6 shows a typical sedi-

pendence of the sedimentation constant on concentration can be established, indicating that the particles are probably not highly asymmetrical. A value of the sedimentation constant for this component has been obtained by taking the average of the values given graphically in Fig. 7, the average of

forty values being $s_{20} = 12.64 S$. The sedimentation constant for the other component from peas shows, however, a distinct dependence on concentration. The sedimentation constant is usually given for infinite dilution, i.e. for zero concentration, and from Fig. 7 the value $s_{20} = 8.10 S$ is obtained.

Attempts to separate the components

In the experiments described above both components have appeared at the same time in the diagrams. Experiments to isolate each component separately have been carried out in order that molecular weight determinations could be made.

buffer and precipitated with 11.0 g. of $(\text{NH}_4)_2\text{SO}_4$. The precipitate was centrifuged off and dissolved in 10 ml. of standard buffer, after which the solution was dialyzed for 2 days. The precipitate obtained was dissolved in 10 ml. of standard buffer. The solution was designated *C* and investigated in the ultracentrifuge.

The results of these precipitation experiments are seen in Fig. 8, which shows that by means of fractional precipitation it is possible to obtain the heavier globulin component relatively pure. The method is not, however, sufficiently effective. It has proved to be very difficult to remove the last traces of the lighter component, even if the method of separation described above is repeated.

Fig. 8. Sedimentation diagrams after fractional precipitation of vicilin and legumin with $(\text{NH}_4)_2\text{SO}_4$. Top left, initial solution; top right, solution *A*; bottom left, solution *B*; bottom right, solution *C* (see text). Ordinates and abscissae as in top left.

Fractional precipitation with ammonium sulphate. The following experiment was carried out: 12.0 g. of $(\text{NH}_4)_2\text{SO}_4$ were added to 25 ml. of 0.6% pea-globulin solution in the standard buffer, with stirring. The precipitate obtained was dissolved in 8.0 ml. of standard buffer (solution *A*). To the centrifugate obtained above were added an additional 3 g. of $(\text{NH}_4)_2\text{SO}_4$ after which the precipitate obtained was centrifuged down and dissolved in 10 ml. of standard buffer (solution *B*). On further addition of salt no more precipitation was obtained.

Both the solutions, *A* and *B*, were dialyzed against distilled water for 2 days at 4° . In this way the globulins precipitated quantitatively. After centrifuging down, the precipitates were dissolved in 10 ml. of standard buffer, and investigated in the ultracentrifuge, whilst the supernatant fluid from *A* (9 ml.) was diluted to 25 ml. with standard

Separation by means of dilution with water at 0° . The behaviour of solutions of pea globulin in standard buffer is strongly dependent on temperature. On cooling down to 0° a milk-white suspension is obtained after about an hour, and if the solution is allowed to stand for a day or two at this temperature, a dense precipitate is obtained on the bottom of the vessel. Preliminary experiments showed that the substance precipitated in this way contained a relatively higher concentration of the heavier component than the original solution. A method of separation based on the above-mentioned properties seemed possible. However, no satisfactory results were obtained merely by cooling the solutions, but instead the solutions were diluted with water at 0° : 10 ml. of 1% pea globulin in standard buffer were cooled to 0° and diluted with distilled water at 0° . At a total volume of 35 ml. (25 ml. of water had been added)

a dense white precipitate was suddenly obtained. An additional 65 ml. of water were added, after which the precipitate was centrifuged down at 0° in a refrigerator. Two fractions were obtained in this way: (A) The supernatant: this was dialyzed against water in a cellophan sac at 4° . After 24 hr. the precipitate obtained was dissolved in 10 ml. of standard buffer and investigated in the ultracentrifuge. (B) The precipitate: this was dissolved in 10 ml. of standard buffer, and the precipitation repeated by the addition of 90 ml. of water at 0° . This was repeated until the substance had been precipitated a total of five times. Finally, the precipitate was dissolved in 10 ml. of standard buffer and investigated in the ultracentrifuge.

Fig. 9. Sedimentation diagrams after separation of vicilin and legumin by means of dilution at 0° . Upper, initial solution; centre, supernatant; lower, precipitate (see text).

The results of the above precipitation experiments are shown in Fig. 9. The heavier component pre-

cipitates to a considerably greater extent than the lighter on dilution at a low temperature. The isolation of the two components is not, however, completely satisfactory if it is desired to prepare solutions for diffusion experiments, and other methods must be employed.

Separation by changing the pH. Separation experiments described earlier have made possible the development of a new, effective method of separation. In a solution containing mainly the heavier component, prepared by fractional precipitation with $(\text{NH}_4)_2\text{SO}_4$ (see Fig. 8), the pH was slowly lowered. At pH 4.5–5 a precipitate was obtained

Fig. 10. Sedimentation diagrams after separation of vicilin and legumin by means of pH change. Upper, initial solution; centre, precipitate at pH 4.5 (legumin); lower, supernatant at 4.5 (vicilin) (see p. 396 of text).

which was soluble on both sides of this precipitation range. In a solution containing the lighter component, prepared by dilution with water in the cold (see Fig. 9), no precipitation occurs at any pH (only the range from pH 3 to 10 has been

investigated here in view of the risk of denaturation). This property has given rise to the most effective method so far for the separation of the two components. A dried preparation, consisting of a mixture of approximately equal portions of the two components, is treated with a large excess of buffer of pH 4.5, with stirring. Acetate buffers containing 0.2M-NaCl have been used. During this procedure the lighter component dissolves, while the other does not. After 24 hr. the heavier component is centrifuged off and dissolved in standard buffer. This solution is dialyzed against water. The centrifugate from the above centrifugation contains the lighter component, and this solution is also dialyzed. After 1-2 days all the globulin has precipitated, and the two precipitates are dried in the cold. On ultracentrifugation of the fractions so obtained the diagrams of Fig. 10 are obtained.

This method of separation, based on isoelectric precipitation of the heavier component, is clearly the most effective of the three methods described here. By repeated use of this method the two components have been completely separated from each other, which makes possible molecular weight determinations of both globulin components.

Comparison with Osborne's globulin components

By isoelectric precipitation two solutions were prepared, one containing the lighter component and the other the heavier. Coagulation experiments were carried out on these fractions, in order, if possible, to obtain some connexion between these two components and the globulin components defined by Osborne (1898). On boiling a solution containing the heavier component, no precipitate was obtained, i.e. the substance does not coagulate on warming to 100°. This component should, therefore, consist of legumin, according to Osborne's definition. On boiling, the component is of course destroyed, which is also shown by ultracentrifugation.

On warming a solution containing the lighter component, precipitation is obtained at 95°, and, on boiling, all the protein precipitates. The lighter globulin component consists, therefore, of vicilin. In what follows the two components will be given these names. Legumin is the component which has a sedimentation constant of 12.64 *S*, while vicilin has a sedimentation constant of 8.10 *S* at infinite dilution.

As mentioned earlier, Osborne has pointed out that vicilin is soluble in more dilute salt solutions than legumin. This is also clearly seen from the separation experiments by dilution with water at 0°. It would seem to be proved by this that the globulin components isolated by Osborne, legumin and vicilin, are identical with the globulins from peas described in this paper. Nothing corresponding to the legumelin defined by Osborne has, however, been detectable on ultracentrifugation.

Determination of the molecular weight of vicilin

The initial solution for determinations of *D* and *V* of vicilin was prepared according to the method described earlier for the separation of vicilin from legumin by isoelectric precipitation of the legumin. This method was applied several times so that the vicilin solution obtained was completely freed from legumin, as was established by ultracentrifugation.

D was determined according to Lamm's (1937) scale method in a stainless-steel cell designed by Claesson (1946), but somewhat modified. The values of *D_m* and *D_A* obtained after different diffusion times are given in Table 5. The agreement between the two

Table 5. *Diffusion constant of vicilin*

(*D* is given in units of 10⁻⁷ c.g.s. at 20°.)

	Diffusion time (hr.)				Average
	23	28	32	46	
<i>D_m</i>	4.44	4.18	4.22	4.30	4.29
<i>D_A</i>	4.31	4.20	4.18	4.17	4.22

different methods of calculation is good. Taking an average value of *D* = 4.26 × 10⁻⁷ c.g.s., and an experimentally determined value of *V*₂₀ = 0.752, the molecular weight of vicilin, using the value of the sedimentation constant found earlier, *s*₂₀ = 8.10 *S*, and *ρ* = 0.9982, is found to be *M* = 186,000.

Determination of the molecular weight of legumin

The initial solution for the determination of *D* and *V* in the case of legumin had been completely freed from vicilin by repeated isoelectric precipitation. *D* was determined in the same cell and in exactly the same way as that of vicilin. The results are given in Table 6. In this case also the agreement

Table 6. *Diffusion constant of legumin*

(*D* is given in units of 10⁻⁷ c.g.s. at 20°.)

	Diffusion time (hr.)					Average
	23	25	28	45	48	
<i>D_m</i>	3.62	3.57	3.52	3.32	3.42	3.50
<i>D_A</i>	3.52	3.39	3.45	3.53	3.53	3.48

between the values of *D_m* and *D_A* is very good, indicating a high degree of monodispersity. For the calculation of the molecular weight of the legumin the values used were: *D* = 3.49 × 10⁻⁷ c.g.s. and *s*₂₀ = 12.64 *S*. *V* was determined, as in the previous case, by Prof. C. Drucker, who obtained the value *V*₂₀ = 0.735. With the value *ρ* = 0.9982, the molecular weight for legumin is found to be *M* = 331,000.

Occurrence of vicilin and legumin in different species of the Leguminosae

The experiments described earlier to isolate seed globulins from different species of the Gramineae supported the working hypothesis that closely related plants contain the same types of protein. A further confirmation of this hypothesis seems to be given by the following investigations on different species of the Leguminosae.

Experiments to isolate the globulins from seeds of different Leguminosae have been carried out in exactly the same way for all the species. Owing to the difficulties in obtaining a sufficient amount of seed of some of the species, the amount of initial substance has varied between 3 and 10 g. The proportion of amount of extraction buffer to amount of seed has been kept constant throughout. The scheme of preparation has been the same as in the experiments described earlier for the isolation of vicilin and legumin from peas, *Pisum sativum*, whereby a solution containing both components is obtained.

Table 7. *Sedimentation constants of the globulin components of various Leguminosae*

(Results in Svedberg units.)

<i>Acacia alata</i>	1.25	7.90	11.63	—
<i>A. decipiens</i>	—	8.02	12.70	—
<i>A. Farnesiana</i>	—	8.04	—	—
<i>A. longifolia</i>	2.72	7.59	—	18.04
<i>A. penninervis</i>	1.46	7.39	—	18.75
<i>A. saligna</i>	—	7.76	13.67	—
<i>A. verticillata</i>	—	7.77	—	—
<i>Arachis hypogaea</i>	1.93	8.40	13.05	—
<i>Astragalus galegiformis</i>	—	8.33	13.17	—
<i>Cytisus Laburnum</i>	—	8.08	14.02	—
<i>C. supinus</i>	1.84	8.03	13.38	—
<i>Dolichos Lablab</i>	—	7.33	11.66	—
<i>Ervum Lens</i>	—	7.25	13.18	—
<i>Genista tinctoria</i>	—	8.54	13.34	20.31
<i>Glycine Soja</i>	—	7.97	13.06	—
<i>Lathyrus Clymenum</i>	—	7.55	13.00	—
<i>L. odoratus</i>	—	7.64	12.00	—
<i>L. sativus</i>	—	7.46	13.04	—
<i>L. silvestris</i>	—	7.48	12.97	—
<i>Lotus Tetragonolobus</i>	—	8.32	13.07	—
<i>Lupinus albus</i>	—	8.24	12.29	—
<i>L. angustifolius</i>	—	8.20	13.05	—
<i>L. luteus</i>	—	8.30	11.53	—
<i>L. polyphyllus</i>	—	8.69	12.20	—
<i>Medicago sativa</i>	—	6.77	11.41	—
<i>Phaseolus coccineus</i>	4.29	7.39	12.16	—
<i>P. nanus</i>	—	6.55	10.10	—
<i>P. vulgaris</i>	4.87	7.26	11.02	—
<i>Pisum sativum</i>	—	8.10	12.64	—
<i>Trifolium hybridum</i>	—	7.66	12.90	—
<i>T. pratense</i>	—	7.69	11.22	—
<i>T. repens</i>	—	7.27	—	18.20
<i>Vicia Faba</i>	—	7.12	11.80	—
<i>V. sativa</i>	—	7.09	11.91	—

As follows from Table 7 and Fig. 11, vicilin and legumin occur in the seeds of practically all the species investigated of the Leguminosae. The most

important exceptions are a few *Acacia* species (*A. longifolia*, *A. penninervis*, and *A. verticillata*). In these species, legumin is absent, while in the other four *Acacia* species it has been possible to isolate legumin, even although in rather small quantities. On comparing *Acacia* with the other species of the Leguminosae it is seen that the former hold a unique position in this family; they are, in fact, sometimes considered as a subfamily within the Leguminosae, and this may be justified to a certain extent by the protein composition of the seeds. Furthermore, it has not been possible to isolate legumin from the seeds of *Trifolium repens*. However, this apparent absence of legumin may simply result from difficulties in preparation, since the various *Trifolium* species are relatively poor in proteins.

It is seen from Table 7 that in some cases globulin components have been isolated with a sedimentation constant of $s_{20} \approx 2 S$. These components probably constitute a degradation product of legumin. According to investigations, as yet unpublished, a component of globulin nature with a sedimentation constant of $1.9 S$ is obtained by dissolving legumin in a buffer of pH 3. The same component is obtained from legumin by protracted heating at 100° . In some cases, a component of $s_{20} \approx 18 S$ has also been obtained, though always in very low concentration; it is found in *Pisum sativum*, but it has not been possible to separate it from vicilin and legumin. Whether it exists in the seeds, or results from complex formation between vicilin or legumin, is not yet clear. Experiments to answer this question seem, however, to indicate the latter possibility.

It is also interesting to compare the relative concentrations of vicilin and legumin in the different species. Closely related species have approximately the same relative concentrations of the two components. In the various *Lathyrus* species, for example, legumin always predominates, as is also the case for the two *Vicia* species investigated. These two genera are, of course, botanically very near to each other. In the various *Lupinus* species it is the vicilin which is in greater concentration, and this also applies to the *Phaseolus* and *Trifolium* species.

If one specially considers *Vicia sativa* it is seen from the sedimentation diagram that vicilin is found in somewhat lower concentration than legumin. This is contrary to the conception of Osborne & Campbell (1898), according to whom vicilin should be completely absent from this species.

In order to obtain more accurate values of the relative concentrations of vicilin and legumin, a new method is being developed for determining the concentrations of these components in solutions containing both, based on the fact that legumin contains about five times more tryptophan than vicilin. By measuring the absorption in the ultraviolet, the

Fig. 11a.

Fig. 11 a-c. Sedimentation diagrams showing the composition of globulin solutions prepared by extraction of seeds from different species of the Leguminosae. Ordinates and abscissae as in top left.

Fig. 11 b. For legend see p. 398.

relative concentrations of vicilin and legumin can be determined. The results will be published in a later paper.

components have been isolated. Different methods have been developed for the separation of these components, which are identical with vicilin and

Fig. 11 c. For legend see p. 398.

SUMMARY

1. The seed globulins of nine different species in the family Gramineae have been investigated. Two well defined globulin components, α and γ , were isolated. The α component was found in six and the γ component in eight species. Seeds of barley contain two other components, β and δ , which were not found in any other species of this family.

2. These components occur in different parts of the seed itself. Investigations on different fractions from seeds of wheat and barley have shown that the γ component alone occurs in the embryo fraction and the α component in the residual fraction. The husk fraction contains γ in higher concentration than α .

3. Determinations of the molecular weight of the purified components have given the values $M = 29,000$ for α and $M = 210,000$ for γ .

4. From the pea, *Pisum sativum*, two globulin

legumin, first defined and investigated by Osborne (1898, 1909).

5. The molecular weight of vicilin was found to be 186,000, and that of legumin 331,000.

6. The seed globulins from thirty four different species in the family Leguminosae have been investigated. Vicilin and legumin were found in all, except in some *Acacia* species and in *Trifolium repens*. In a few cases degradation products and complex compounds of these components have been isolated.

7. These investigations seem to confirm the hypothesis that closely related plants contain the same seed globulins.

The author wishes to thank Prof. Svedberg for the great and stimulating interest which he has shown in this work, and for the privilege of working in his laboratory. The expenses connected with these investigations were defrayed by the AB. Stockholms Bryggerier and the Rockefeller Foundation.

REFERENCES

- Claesson, S. (1946). *Nature, Lond.*, **158**, 834.
 Czapek, F. (1905). *Biochemie der Pflanzen*, **2**, 157. Jena: Fischer.
 Lamm, O. (1937). *Nova Acta Soc. Sci. upsal.* (Ser. IV), **10**, 6.
 Osborne, T. B. (1898). *J. Amer. chem. Soc.* **20**, 348.
 Osborne, T. B. (1909). *The Vegetable Proteins*. London: Longmans, Green & Co.
 Osborne, T. B. & Campbell, G. F. (1898). *J. Amer. chem. Soc.* **20**, 410.
 Quensel, O. (1942). *Untersuchungen über die Gerstenglobuline*. Dissertation. University of Uppsala, Sweden.
 Säverborn, S., Danielsson, C. E. & Svedberg, T. (1944). *Svensk kem. Tidskr.* **56**, 75.
 Svedberg, T. & Pedersen, K. O. (1940). *The Ultracentrifuge*. Oxford: Clarendon Press.