124. PROTEIN METABOLISM IN SEED GERMINATION

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FEW aspects of plant physiology have excited an interest comparable with that manifested for many decades in the protein metabolism of germination. A modern interpretation of the earlier work and a correlation of it with recent research have been given by Chibnall [1939]. Our knowledge of the changes in protein composition which accompany germination still, however, largely lacks the support of direct experiment.

In recent years claims have been renewed that germinated seeds, or seedlings, are frequently of greater value in stock feeding than the ungerminated seeds; see, for example, the review by Leitch [1939]. In regard to palatability, digestibility, vitamin content, biological value of the protein etc., these claims may well be justified in some instances. It has long been known, however, that with some seeds an enormous loss of protein N and corresponding gain of non-protein N, accompany the transition from seed to seedling. Thus seeds containing much protein and but little fat or carbohydrate are of this type, as Schulze [1880] and others have shown, and a large fraction of the non-protein N produced usually occurs in asparagine. In the event of the total seedling protein being of higher biological value than that of the seed, the question remains whether there has been a net gain or loss of those amino-acids, contained in the proteins, which are dietary essentials. The non-protein N substances produced must be accorded nutritive value, particularly if they should happen to consist not so much of the amides, asparagine and glutamine, but of a relatively unchanged assemblage of amino-acids split from the seed protein by zymolysis.

In this article data will be presented giving direct information on some of the points raised above. The work was done with the blue lupin, *Lupinus angustifolius*, seedlings of which were being examined as a source of asparagine. Seedlings of the yellow lupin, *L. luteus*, might have been a better source, for as Schulze [1898] showed, the seeds of this plant have a lower carbohydrate plus fat to protein ratio than seeds of *L. angustifolius*. The latter were used because they were available in quantity.

EXPERIMENTAL

Preparation of materials—N balance

The seeds, which showed about 96% viability, were germinated on wet, purified cotton wool, and the seedlings were cultured on water in virtual darkness at about 27°. 14 days after setting the seeds, that is, about 12 days after the emergence of the rootlets (a few emerged in the first day and a few late ones in the third day), the crop from 110 germinated seeds was harvested.

The testas were discarded and the seedlings (*c*) were divided into cotyledons (*d*) and axial organs (*e*), and these latter in turn were divided into roots, or the portions of the hypocotyls which had been submerged in the water (*g*), and stalks with their young plumules (*f*). Another 110 ungerminated seeds taken at random were divided into testas (*a*) and embryos (*b*); see Table 1.

Table 1. *Germination of 110 seeds of Lupinus angustifolius*

Fresh wt. 26.55 g., dry wt. 23.78 g. Seedlings harvested 1. viii. 1940.

	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(c)-(b)
	Testas	Embryos	=(d)+(e)	Coty- ledons	=(f)+(g)	Stalks + plumules	Roots	Net gain
Fresh wt. (g.)	6.60	19.95	209.1	44.7	164.4	143.6	20.8	189.1
Dry wt. (g.)	6.10	17.68	14.70	5.22	9.48	8.13	1.35	-2.98
(% N, on dry wt.)	(0.80)	(7.12)	(8.56)	(6.61)	(9.63)	(10.04)	(7.18)	—
Total N (g.)	0.049	1.258	1.258	0.345	0.913	0.816	0.097	0.000
Coagulable N (g.)	—	1.095	0.279	0.139	0.140	0.105	0.035	-0.816
Protein N (g.)	—	0.976	0.259	0.132	0.127	0.094	0.033	-0.717
Dry wt. protein prep. (g.)	—	10.95	—	2.30	3.21*	2.53	0.68	—
(% N in dry protein prep.)	—	(8.91)	—	(5.74)	(3.96)	(3.70)	(4.88)	—

* By addition of (*g*) and (*f*).

Samples of these materials were taken for moisture and total N estimations. The main bulks (except testas (*a*) on which no further work was done) were treated as described in other articles [Lugg, 1938, 2, 3] for removal of non-coagulable and other non-protein nitrogenous substances, by macerating, and extracting with hot water at pH 4.5 (the residue at this stage containing 'coagulable N'), followed by alcohol, dilute Na citrate solution at pH 3.5 and ether (the residue at this stage containing only 'protein N'). As the initial coagulation was not assisted by addition of alcohol, several % of the protein N of (*f*), (*g*) and (*d*) may have gone into the non-coagulable N fractions [Lugg, 1939]. The dried materials remaining after extraction constituted crude protein preparations. All N estimations were made by the Kjeldahl method. The data are shown in Table 1. The main bulks of the crude protein preparations were kept in reserve after those from (*g*) and (*f*) had been mixed in the proportion necessary to represent the axial organs (*e*). The analyses of these preparations are described later.

The total N in the 110 seedlings was 2.8% lower than that in the 110 ungerminated embryos. There was no visually obvious correlation between size of seed and viability, and in any case viability was high. The probability of a 2.8% or greater difference in total N content between any two groups of 110 seeds taken at random, was calculated as 0.36 from a statistical analysis of data for ten such groups. It has been assumed that there was neither assimilation of atmospheric N, transfer of N to or from the testas nor discharge of seedling N into the water. The lower N content of the seedlings has been attributed solely to random sampling errors, and the corresponding corrections have been made in Table 1 to show no net gain or loss of total N.

From the non-coagulable material in the stalks and plumules (0.711 g. N), 2.56 g. of recrystallized asparagine monohydrate (0.477 g. N) were obtained.

Partial analyses of the protein preparations

The amide, tyrosine, tryptophan, cystine plus cysteine and methionine contents of the protein preparations from ungerminated embryos (*b*) and from the cotyledons (*d*) and axial organs (*e*) of the seedlings, were estimated.

The methods used, estimation of NH_3 liberated by mild hydrolysis with acid for amide; colorimetric estimations after alkali-stannite hydrolysis for tyrosine (diiodotyrosine and dibromotyrosine were presumed to be absent from the preparations) and tryptophan, and sulphate estimations following differential oxidation for cystine plus cysteine and methionine, have been described in detail in other articles [Lugg, 1937; 1938, 1, 2, 3] wherein their applicability to crude protein preparations has been demonstrated.

Some difficulty was encountered in the tryptophan estimations, as the tryptophan contents were rather small and there were fairly large corrections for adventitious coloration. Incidentally it is best, when concentration of a prepared acidified hydrolysate before mercuration is desirable, to render the hydrolysate alkaline and evaporate at fairly low temperature under reduced pressure. But this entails increase in the ultimate sulphate content, and it has been found satisfactory in certain cases (some leaf-protein preparations) to distil water from the acidified hydrolysates at temperatures up to 45° [Lugg, 1938, 1]. It is safer to evaporate at temperatures up to 25° , and this is conveniently done by exposing at low pressure over H_2SO_4 in a vacuum desiccator. At the higher temperature there was evidence of loss of tryptophan in the case of the lupin embryo protein preparation, due presumably to production of aldehydes and their condensation with some of the tryptophan.

In Table 2 are recorded the partial compositions of the protein preparations. Corrections have been made for such systematic errors as are revealed when the pure amino-acids are put through the analytical processes. For discussion of

Table 2. *Partial compositions of the proteins*

Protein prep.	% N in prep.	% Amide N	% Tyr. N	% Try. N	% Cyst. N	% Meth. N	% Cyst. + meth. N	$\text{SO}_4\text{-S/N}$ $\times 100$	% Ash in prep.
(b) Embryos	8.91	9.82	1.70	0.62	1.16	0.24	1.40	0.002	1.1
(d) Cotyledons	5.74	7.43	2.21	0.79	1.43	0.59	2.02	0.007	1.5
(e) Axial organs	3.96	5.35	2.24	0.91	1.09	1.03	2.12	0.15	1.7

other possible systematic errors (assumed to be zero in the forthcoming discussion) reference may be made to the articles describing the methods. The cystine plus cysteine and methionine estimations have been based upon the assumption that all the S in the protein preparations may be accounted for in these amino-acids and in sulphate (either inorganic or ethereal). The random errors of estimation are believed not to exceed the amounts shown in parenthesis in the following description of the contents of the Table. In the order of the columns are: the label of the preparation, the percentage of N in the dry preparation (0.5 %, affecting the other errors), the amide content (1.5 %), tyrosine content (2 %), tryptophan content (5 %), cystine plus cysteine content (2 %), methionine content (4 % for (e), 6 % for (d) and 8 % for (b)), the sum of the last two values (2 %), sulphate-S as a percentage of the weight of N in the preparation (10 % for (d) to 100 % for (b)), and finally the percentage of ash in the preparation after ignition in air at about 600° . The amino-acid and amide contents have been reported on a N basis, that is, as percentages of the protein N occurring in these forms. They are mostly the means of duplicate or triplicate estimations, and some of these had to be made on a smaller scale than usual because only small amounts of material were available. Values in the last two columns are to be taken as measures of various impurities.

DISCUSSION

As Table 1 shows, there was a loss of 73.5 % (0.717 g.) of protein N during the transition of the lupin embryos to 14-day seedlings. But the gain in non-coagulable N was 0.816 g. The difference between the coagulable N and protein N of the embryos (0.119 g.) is probably to be attributed to the presence of considerable lipin. It is to be noted that to an even greater extent than the protein N, this was converted into non-coagulable forms by germination.

No attempt was made to obtain asparagine from the cotyledons or roots. The amount separated in recrystallized form from the non-coagulable material in the stalks with plumules, represented 67 % of the N in that material, 49 % of the non-coagulable N in the entire seedlings and 38 % of the total N in the seedlings or in the embryos.

Some points of considerable interest emerge from Table 2. It will be noticed that the protein of the embryos had a fairly low tryptophan content and a distinctly low methionine content. Osborne & Campbell [1897] found that the various proteins in the embryos of *L. angustifolius* all contained about 18 % of N when carefully purified, and if it be assumed that the mixed embryo proteins in a pure state contained 18.0 % N, a simple calculation gives 0.81 % tryptophan and 0.46 % methionine in the embryo protein on a weight basis.

From the standpoint of animal nutritional requirements, it seems from the work of Bauguess & Berg [1934] that a tryptophan content no higher than this might render the lupin embryo protein somewhat unsatisfactory at a low level of intake. And from the work of White & Beach [1937-8], who found the very poor value of arachin as a rat food to be associated with its low methionine content (0.54 % according to Baernstein [1932]), and that of Womack *et al.* [1937], it must be concluded that in spite of a moderately high cystine content the low methionine content renders the lupin embryo protein very unsatisfactory.

The protein of the axial organs appears, from these analyses, to have been approaching in composition those of the photosynthesizing leaves of leguminous plants, and it is possible that the composition of the protein of the etiolated plumules themselves may have corresponded quite closely with that of the protein of photosynthesizing leaves of this plant. For the plumules were small, and although they were presumably relatively rich in protein (Schulze & Castoro [1903] found them to be so in the case of *Lupinus albus*), a considerable part of the protein in the axial organs must have been present in the stalks and roots (26 % is known to have been present in the roots) and there is at least meagre evidence that the protein in petioles may not correspond extremely closely in composition with that in the leaves they support [Lugg, 1940].

Recent work on the whole leaf proteins of legumes [Lugg, 1940, and unpublished data] suggests that the earlier analyses of samples of protein extracted from legume leaves [Chibnall, 1939; Lugg, 1938, 3] would be reasonably valid for the whole protein of those leaves. It was pointed out that many of the tryptophan estimations in the earlier analyses [Lugg, 1938, 3] were vitiated by the presence of interfering indoles. This difficulty has not been encountered in the more recent work. On the basis of what is now known of the composition of the leaf proteins of legumes generally, the protein of the lupin axial organs contained about 100 % of the amide N which might be expected in the protein of the green leaves of this plant, about 90 % of the tyrosine N, about 60 % of the tryptophan N, about 90 % of the cystine plus cysteine N and about 80 % of the methionine N.

The composition of the protein of the cotyledons suggests that it too was approaching the composition which might be expected of the protein in the

green leaves of this plant. The amide content was high whereas the tryptophan and methionine contents were both low, and the impression is gained that the cotyledon protein was something of a compromise between those of the embryos and axial organs in composition.

When seedlings with epigeal cotyledons are grown in the light, the cotyledons, like the plumules, become green and may be presumed to have a photosynthesizing function. They might therefore be expected to contain proteins characteristic of the normal photosynthesizing tissues as well as reserve seed proteins not yet used up. The amide, tryptophan and methionine contents of the lupin cotyledon protein lie in such a position between those of the proteins of the embryos and axial organs, as to suggest that in terms of protein N, the cotyledons contained about as much axial organ as embryo protein. But the tyrosine content of the cotyledon protein was only doubtfully lower than that of the axial organ protein, and the cyst(e)ine content was actually somewhat higher than that of the protein of either the embryos or axial organs. Among possible explanations are (1) that the different proteins originally present in the cotyledons may have been used up at different rates, (2) that even if the rates were similar, the proteins may have been degraded stepwise and if cyst(e)ine residues, for example, were rather late in being removed, degraded protein relatively rich in cyst(e)ine could have resulted, and (3) that (1) and (2) may have operated together. But in regard to degraded proteins there is the unanswered question of the stage of degradation or synthesis at which the methods used in this experiment would permit such protein to enter the category of 'non-coagulable nitrogenous substances', and for this reason, if for no other, it is profitless to speculate further on the nature of the cotyledon protein.

It remains to discuss the gain or loss by germination of amide and amino-acid residues contained in protein. Table 3 has been drawn up for this purpose

Table 3. *Retention and regeneration of protein components in the proteins of the 110 seedlings*

	(b) Embryos	(c) = (d) + (e) Seedlings	(d) Cotyledons	(e) Axial organs	(c)-(b) Net gain	100 (c)/(b) % Retention + regeneration
Protein N (mg.)	976	259	132	127	-717	26.5
Amide N (mg.)	95.8	16.6	9.8	6.8	-79.2	17.3
Tyrosine N (mg.)	16.6	5.8	2.92	2.84	-10.8	35
Tryptophan N (mg.)	6.1	2.2	1.04	1.16	-3.9	36
Cyst(e)ine N (mg.)	11.3	3.3	1.89	1.38	-8.0	29
Methionine N (mg.)	2.3	2.1	0.78	1.31	-0.2	90
Cyst. meth. N (mg.)	13.7	5.4	2.67	2.69	-8.3	39

from the data in Tables 1 and 2, and gives, among other things, a comparison of the amounts of amide N and amino-acid N contained in the protein of the embryos with those contained in the proteins of the seedlings (by addition of the amounts contained in the proteins of the cotyledons and axial organs). The last but one column in Table 3 shows the net 'gains' resulting from germination. They are all negative. The last column shows the percentage retention plus regeneration of the protein components.

Whereas of the original protein N, 26.5% could be accounted for in the seedlings, only 17.3% of the amide N could be so accounted for, but more than 26.5% of each of the other components. The most interesting case is that of methionine which, alone of the components, shows anything like complete retention plus regeneration. It is regrettable that the value shown (90%) is

subject to random errors of the order of 10%. It is possible too that of the seedling protein several % may have escaped flocculation and coagulation, as mentioned earlier. Consequently the protein methionine balance cannot be very accurately known. The loss may have been somewhat greater than 10% or there may have been a gain of several % by synthesis. If the results be taken to indicate that the supply of original protein methionine may have been a limiting factor in the regeneration of seedling protein, then, as considerable cyst(e)ine was not used up in the regeneration, this would probably imply that the etiolated seedlings, unlike mature plants growing in the light, were unable to synthesize methionine.

There was scant provision for possibilities of this sort in the theories of protein regeneration advanced by the earlier workers. The inability of etiolated seedlings of this type to develop beyond a certain stage and the piling up of amides and other non-coagulable nitrogenous substances instead of regenerated protein, were attributed in the theories of Schulze [1898] and Prianischnikow [1899] to lack of suitable carbohydrates or other non-nitrogenous metabolites. It is true that Prianischnikow, in opposition to Schulze, considered the seed protein amino-acids to play a primary part and the amides a secondary one in protein regeneration, but in his theory the first limitation upon regeneration occurs when, by lack of other metabolites, the seed protein or amino-acids have to supply the seedlings' energy requirements. Chibnall [1939] insisted that Paech's [1935] hypothesis concerning the breakdown and synthesis of protein in plant tissues becomes more acceptable if the factor 'active carbohydrate' is replaced by ' α -keto-acids', and pointed out that even when ample carbohydrate and asparagine (as a source of N) are present, a failure at any of the steps in the reactions: sugar $\rightarrow$ α -keto-acid $\rightarrow$ α -amino-acid $\rightarrow$ protein, could limit protein regeneration. Thus, although Chibnall did not discuss the possibility of the regeneration being limited by the supply of specific seed-protein amino-acids, failure at some step in a chain of reactions similar to the one he described, but involving S as well as N, might limit the supply of methionine for protein regeneration to the amount existing in the seed protein. And if that amount were very small it could be a first limiting factor in protein regeneration.

No whole leaf proteins are known to be nutritionally deficient, and the lupin seedling protein was probably of much higher biological value than that of the embryos. But the amounts of most of the dietary essential amino-acids, contained in protein, were probably reduced by the transition from seed to seedling (64% of the tryptophan, for example, was missing) and it is highly improbable that the amounts lost in this way could have been fully present among the non-coagulable substances, because the asparagine production was very large and must have involved the metabolism of large quantities of amino-acids. For feeding purposes a greatly increased degree of protein regeneration would have been desirable. Such advantages as may exist in the feeding of seedlings instead of seeds would probably be increased, with seeds of this type, by culturing for much longer periods and in light instead of darkness.

SUMMARY

Etiolated seedlings, in which more than 38% of the N was present as asparagine, were grown from seeds of the blue lupin, *Lupinus angustifolius*. The retained plus regenerated protein N represented only 26.5% of the protein N of the embryos. The proteins in the embryos and in the cotyledons and axial organs of the seedlings, contained respectively (on a N basis): 9.82, 7.43 and 5.35% amide N; 1.70, 2.21 and 2.24% tyrosine N; 0.62, 0.79 and 0.91%

tryptophan N; 1.16, 1.43 and 1.09 % cyst(e)ine N; and 0.24, 0.59 and 1.03 % methionine N.

From these analyses the axial organ protein appears to have been approaching in composition those of the leaves of legumes. The cotyledons probably contained protein of similar composition mixed with incompletely broken down embryo protein. In the protein of the seedlings, it was possible to account for 17.3, 35, 36, 29 and 90 %, respectively, of the amide N, tyrosine N, tryptophan N, cyst(e)ine and methionine N, originally present in the embryo protein.

It has been pointed out that the embryo protein must be of low biological value on account of its very low methionine content, and that whereas the seedling protein was probably of much higher biological value, germination probably resulted in a net loss of most of those amino-acids, present in protein and among the non-protein nitrogenous substances, which are dietary essentials.

The possibility has been discussed of the low methionine content of the embryo protein imposing a limitation upon protein regeneration in the etiolated seedlings.

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