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Protein in Senescent Leaves of *Trifolium subterraneum*: Partial Amino-acid Composition

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It has been shown (Lugg & Weller, 1941) that in very young leaf tissue the whole protein composition may differ rather markedly from that of the whole protein in older tissue. (The axial organs of germinating seeds were used, but the bulk of the protein was present in the stalks and plumules.) In the following account it will be shown that at the other extreme of age (senescent tissue) the departure in composition of the whole-leaf protein from the pattern which seems to prevail during most of the life of the plant may again be fairly marked in some respects.

EXPERIMENTAL

Preparation of materials. The fresh plant material consisted of the leaves and attached petioles of *Trifolium subterraneum* harvested very late in the season (December, 1940) from senescent plants growing in a relatively cool and damp environment in the Adelaide foothills. 'Yellowing' was somewhat advanced in about a third of the leaf material, and much of the rest showed traces of it.

From the bulk of the fresh material an 'extracted' protein preparation (E) was made by the alkaline buffer lipid-

solvent method (Lugg, 1939) in the manner described by Lugg & Weller (1944). 'Residual' (R) and 'whole' (W) protein preparations were also made by methods previously described (Lugg, 1939).

Data concerning the fresh material and the protein preparations made from it are shown in Table 1. The terms 'coagulable N' and 'protein N' have been explained fully in earlier publications (e.g. Lugg, 1939). For the rest, the table is self-explanatory.

Analytical work. Nitrogen estimations were made by the Kjeldahl method with Se catalyst. The amide, tyrosine, tryptophan, cyst(e)ine (i.e. cysteine plus cystine) and methionine contents of the protein preparations, W, E and R, were estimated by methods previously described (Lugg, 1937; 1938a, b, c; Lugg & Weller, 1941). The 'differential oxidation method' was used for the estimation of the cyst(e)ine and methionine contents. Considerable difficulty was encountered with the tryptophan estimations on the W and R preparations because of the unusually intense brown coloration of the alkali-stannite hydrolysates. Corrections for this coloration were so large that the precision of the estimations was seriously impaired.

The arginine, histidine and lysine contents of preparation E were estimated by methods recently described (Lugg & Weller, 1948) and based upon the procedures of Tristram (1939) and of Vickery & Winternitz (1944). These methods are considered to be unsatisfactory for use with preparations

Table 1. *Extraction of protein from senescent leaves of Trifolium subterraneum*

Fresh wt. taken (kg.)	Dry wt. (%)	N in dry leaf (%)			Extracted N (%)			Protein pre- paration	N in prep. (%)	Ash in prep. (%)
		Total	Coagulable	Protein	Total	Coagulable	Protein			
12.3	20	2.45	2.05	1.99	—	—	—	W	3.16	2.4
—	—	—	—	—	33	28	26	E	13.15	2.6
—	—	—	—	—	—	—	—	R	2.10	3.5

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like W and R. Of the protein N, there were estimated to be present in the hydrolysates, 11.2% by method 1 and 11.0% by method 2 (11.1% mean) as arginine, 4.07% by method 2 as histidine and 5.72% as lysine. In deriving this lysine value, weights of crude lysine monpicrate were corrected by multiplying them by the factor 0.975 determined previously for use in analyses of other leaf-protein preparations (Lugg & Weller, 1948). The arginine and histidine values required correction for losses attributable to the presence of interfering impurity in the protein preparation. The interfering impurity in 2.5 g. of preparation E was calculated from the N and ash contents of the preparation and assumed N content of 16.75% for the hypothetical pure protein (see Lugg & Weller, 1948) to correspond in quantity and effects with 0.55 g. of arabinose, and the above arginine and histidine values were accordingly multiplied by the appropriate factors (see Lugg & Weller, 1948).

RESULTS

The results are shown in Table 2, the partial compositions of the preparations being expressed as percentages of the protein N occurring in amide and

amide, tyrosine, tryptophan, cyst(e)ine, methionine and cyst(e)ine plus methionine contents of the preparations, and of the rationale of the method of obtaining preparation E, it is believed that this preparation was reasonably representative of the whole protein in the leaf material.

Viewed broadly, the leaf protein composition appears to be similar to those found for other spermatophytes. Comparison of the contents of Table 2 may be made with those of tables in recent publications (Lugg & Weller, 1948; Lugg, 1946). A significant feature of these other tables is that the cyst(e)ine and methionine contents of the whole proteins in leaf tissue from reasonably mature plants are, in general, somewhat lower for the Leguminosae than they are for any of the other plant families investigated. For 'whole', 'extracted' and 'residual' protein preparations from *Trifolium subterraneum* specifically, Lugg (1943) found the cyst(e)ine-N values to be 1.15, 1.13 and 1.12%, respectively, and the methionine-N values to be 1.32,

Table 2. *Partial compositions of protein preparations from Trifolium subterraneum*

Preparation	N (%)	Percentages of protein N								
		Amide N	Tyrosine N	Tryptophan N	Cyst(e)ine N	Methionine N	Cyst(e)ine + methionine N	Arginine N	Histidine N	Lysine N
W	3.16	5.48	2.80	1.85	1.42	1.05	2.47	—	—	—
E	13.15	5.39	2.81	1.80	1.43	1.13	2.56	13.5	4.26	5.72
R	2.10	5.71	2.90	1.65	1.47	1.02	2.49	—	—	—

the amino-acids concerned. Except for the tryptophan contents the random errors with the W and R preparations are believed not to have been more than 1.5 times as great as those with preparation E, and these in turn are believed not to have exceeded the following percentages (in parentheses) of the tabulated values: N (0.5, affecting all the other errors), amide (1.5), tyrosine (2), tryptophan (3), cyst(e)ine (4), methionine (5), cyst(e)ine plus methionine (2), arginine (4), histidine (2) and lysine (8). Estimates of the likely limits of random errors in the arginine, histidine and lysine values have been based partly upon the deviations encountered in the present work and partly upon those encountered in earlier work with these methods (Lugg & Weller, 1948). Those for the other values have been derived from much experience with the methods concerned.

DISCUSSION

In view of the rather low protein content of the leaf material the yield of 'extracted' protein was not, for the procedure employed, unduly poor, nor was the purity of the preparation (judged from its N content) unexpectedly low. Presumably, therefore, no large fraction of the leaf protein was in a denatured condition. From a consideration of the

1.38 and 1.29%, respectively, the cyst(e)ine-N plus methionine-N values being 2.47, 2.51 and 2.41%, respectively. The cyst(e)ine-N plus methionine-N values for preparations W, E and R of the present study are much the same as these, but it will be noticed that the cyst(e)ine contents are distinctly higher whilst the methionine contents are distinctly lower than those reported for the non-senescent material. Indeed, the cyst(e)ine contents are above the range of values, and (except for the seedling material examined by Lugg & Weller, 1941) the methionine contents below the range of values which have been estimated by this method for all other legume leaf materials. Again, the tyrosine values for preparations W, E and R, which are somewhat higher than those reported for the non-senescent material (Lugg, 1943), are among the highest yet encountered in leaf-protein preparations. The histidine content of preparation E is slightly higher than those of two legume- and two grass-leaf preparations recently examined (Lugg & Weller, 1948).

From past studies of plant-leaf protein preparations we have come to associate small, and usually rather uncertain, composition changes with different samples of leaf material harvested at different times during most of the life history of the plant. We do not know whether the protein in senescent tissue of

T. subterraneum would always correspond closely in composition with the amide and amino-acid values we have obtained in this one instance. In so far as it may be safe to generalize from the data at present available, the onset of senescence appears to have been accompanied by an increase in the cyst(e)ine content (and possibly in the tyrosine content) and a decrease in the methionine content of the whole protein in the leaf material. We do not know whether the compositions of individual proteins in the leaves have undergone change. It is possible that the only change has been in the proportions of the individual proteins. In this connexion it is pertinent to point out that change in haemoglobin composition with transition from foetal to adult mammalian life (as shown by Vickery (1944) for the histidine contents of foetal and adult bovine haemoglobins) may be due to a change in the ratio of two (or more) haemoglobin components (demonstrated for foetal and adult human material by Andersch, Wilson & Menten, 1944).

Effects of plant age and treatment (such as grazing) upon the cyst(e)ine and methionine contents of leaf proteins were judged by Smith & Wang (1941) to be fairly pronounced, but the validity of their conclusions was questioned by Lugg (1943) on the ground that the analytical methods they used would be highly unreliable with the impure leaf-protein preparations they analyzed. That changes in

the cyst(e)ine and methionine contents of the whole protein in the leaves of various plants may occur, particularly at the extremes of plant age, seems very likely, but our earlier work, covering considerable ranges of the intermediate ages of plants, has revealed no definite, progressive changes.

SUMMARY

1. The amide, tyrosine, tryptophan, cyst(e)ine, methionine, arginine, histidine and lysine contents of protein preparations from senescent leaves of *Trifolium subterraneum*, expressed as percentages of the protein N occurring in these forms, were found to be 5.39–5.71, 2.80–2.90, 1.65–1.85 (best 1.80), 1.42–1.47, 1.02–1.13, 13.5, 4.26 and 5.72, respectively.

2. From comparison of these values with those previously found for non-senescent material (arginine, histidine and lysine values were not available) and from other considerations, it has been concluded tentatively that the onset of senescence was accompanied by an increase in the cyst(e)ine and possibly in the tyrosine content, and a decrease in the methionine content of the whole protein in the tissues.

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Nicotinic Acid in Cereals

1. THE EFFECT OF GERMINATION

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Nutrition surveys have shown that under present conditions dietary supplies of nicotinic acid are being obtained largely from cereals. These vary widely in their nicotinic acid content. Maize and oats are poor sources, rice and barley are better and whole wheat is probably the best of the staple cereals.

Kodicek (1940) found that chemical methods gave higher results for maize than biological methods, and suggested that better agreement might be obtained if extraction were carried out with hot water instead of acid or alkali. Melnick (1942), reporting the results of a collaborative study, stated that with acid extraction chemical methods gave higher results than