185. TISSUE PROTEINS OF SOME CRYPTOGAMS: THE AMIDE, TYROSINE AND TRYPTOPHAN CONTENTS

BY JOSEPH WILLIAM HENRY LUGG

*From the Nutrition Laboratory of the Division of Animal Health and
Nutrition, Council for Scientific and Industrial Research, at the
University of Adelaide*

(Received 26 October 1940)

FROM the standpoint of biological evolution it might be conjectured that some modification in the amino-acid compositions of tissue proteins would accompany evolutionary development, but it might be argued contrariwise that a pronounced change in composition could be anticipated only in association with a change in function of a tissue. Correspondingly, among the present day descendants of a common ancestor, according to their degrees of development from, or retention of, the primitive characteristics, one might expect variation in the protein composition or anticipate constancy.

Extensive analyses of animal proteins have been published and although many of them require repetition by newer and more critically examined methods, certain regularities have been established soundly enough, e.g. the high cystine contents of the proteins of epidermal structures like hair, wool, horn etc., and the high arginine contents of the protamines. Block & Vickery [1931], and Block [1934] and in subsequent articles, have drawn attention to the constancy of the ratio of the histidine, lysine and arginine contents of proteins in groups arranged according to biological origin, physical properties etc. The results of Bailey's [1937] partial analyses of muscle proteins may be expressed briefly as follows: such evolutionary developments as can be traced through the crustaceans, fish, birds and mammals have probably been without marked influence upon the compositions of the skeletal muscle myosins. It would seem, in general, that the proteins of animal tissues having similar functions will have similar physical properties and will be similar in composition.

Comparative studies of plant proteins have generally been confined to the seeds of the angiosperms, chiefly on account of the ease with which the proteins could be obtained reasonably free from other classes of organic material. The more recent analyses have sustained the conclusions drawn from the earlier work, that between seed proteins the differences in composition are frequently very large but that marked regularities do exist, as in the high proline and glutamic acid contents of the gliadins of seeds of the Gramineae.

It is only in recent years that detailed knowledge of the compositions of proteins of the leaves of these higher plants has been acquired. As reviewed by Chibnall [1939], the analyses show remarkable uniformity of composition, almost comparable with the degree of uniformity among mammalian myosins. There are differences as between the Leguminosae, Gramineae etc. but they are not large.

The main purpose of this article is to record partial analyses of the proteins of somatic, photosynthesizing parts of some lower land plants, and to compare

the results with those for the higher plants. Results obtained with *Trifolium subterraneum*, a legume of considerable economic importance in Australia, are included.

Preparations

Freshly harvested plant tissues were used. The thalli of *Lunularia cruciata* were freed from rhizoids and gemmae. The pinnae of *Pteridium aquilinum* were stripped from the petioles and were selected free from sori. The leaf and stem material of *Selaginella* sp. (probably *Jungermannioides* Group) was free from stroboli and root-bearing branches. Juices from crushed samples of these tissues were found to be of pH 6.0, 5.2 and 5.5 respectively. Only the leaves of *Trifolium subterraneum* were macerated for extraction of protein, the fresh-weight ratio of leaves to petioles was 1 : 1.2, and the dry-weight ratio was 1 : 0.7.

The protein preparations are listed in Table 1 in the order, from top to bottom, of increasing evolutionary development of the plants. In the order of the columns are shown: the label of the protein preparation, viz. a number

Table 1. *The plant materials and protein preparations*

Prep.	Source and date	% dry wt.	% N in dry tissue (t, c, p)	% extracted or residual N (t, c, p)	% N in prep.	% ash in prep.
1 W	<i>Lunularia cruciata</i> [thalli] x. 39-vi. 40 (liverwort)	7.1	3.85t, 2.41c, 2.27p	—	3.92	3.5
2 W	<i>Pteridium aquilinum</i> [pinnae] 7. vi. 39 (bracken)	18.7	4.32t, 3.80c, 3.69p	—	5.82	1.6
2 E	Do.	—	—	69.9t, 69.9c, 69.3p	11.10	1.4
2 R	Do.	—	—	30.1t, 31.5c, 31.5p	2.70	1.6
2 E 1	Do.	—	—	46.5t, 42.1c, 41.8p	12.00	1.3
3 W	<i>Selaginella</i> sp. [leaves and stems] 24. x. 39 (smaller club-moss)	13.6	2.32t, 1.92c, 1.82p	—	3.12	9.9
4 W	<i>Trifolium subterraneum</i> [leaves] 10. xi. 39 (subterranean clover)	18.8	5.10t, 4.26c, 4.12p	—	7.17	0.7
4 E	Do.	—	—	38.7t, 34.4c, 33.3p	14.10	0.4
4 R	Do.	—	—	61.3t, 66.3c, 65.8p	5.26	3.1
4' W	<i>Trifolium subterraneum</i> [petioles] 10. xi. 39 (subterranean clover)	10.8	1.84t, 1.38c, 1.30p	—	2.74	0.7

followed by an abbreviation, W, E or R indicating whether "whole", "extracted" or "residual"; the species of plant and type of tissue, with date of harvest; the percentage dry weight of the tissue; the percentage total (t), coagulable (c) and protein (p) N in the dry tissue; the percentage of the tissue-N (t, c and p) appearing in an extracted juice or tissue residue; the percentage N in the dry protein preparation; and finally, the percentage ash in it by ignition in air at about 600°.

The extracted preparations were made by methods discussed earlier [Lugg, 1939], in which and previous publications [1938, 2, 3], the meanings of terms such as "coagulable N", "protein-N", etc. have been discussed fully.

Preparation 2E was made by macerating the pinnae fairly finely in a power-driven mincer with a borate buffer (pH 9), sieving the mash, centrifuging the liquid for a minute or so in a field of 200 × gravity, and flocculating and coagulating the protein by bringing the juice to about pH 4.5, adding 0.7 vol. of alcohol and heating to about 75°. Preparation 2E1 was made by treating an aliquot of the unflocculated juice (adjusted to pH 9.2) with a cooled mixture of 0.8 vol. of alcohol and 0.2 vol. of ether and filtering the liquid off after 15 min.

through a pad of paper pulp before flocculating and coagulating the protein. The data have been calculated back as if all the juice had been used for each preparation, 2E and 2E1. Preparation 4E was made by coarsely macerating the leaves in the mincer with a pH 9 borate buffer to which had been added 0.8 vol. alcohol and 0.2 vol. ether. The mash was sieved and centrifuged at $1000 \times$ gravity for 30 min. before flocculating and coagulating the protein. The coagula were extracted as usual with hot alcohol, hot dilute citric acid solution (or Na citrate at pH 3.5), hot alcohol again and finally warm ether. Values recorded in Table 2 have been adjusted for the small errors, one way or the other, to show perfect recovery of tissue total N between residue and juice (except of course, in the case of 2E1 where material was retained by the filter pad). Preparations 1W, 2W, 3W and 4W were made by extracting the non-protein-N from samples of the powdered dry tissues as described by Lugg [1939], and 2R and 4R were obtained from the tissue residues after extraction of 2E and 4E respectively, by removing the non-protein-N.

It was anticipated, from the work with grasses [Lugg, 1939], that 2E1, by virtue of the method of preparation, would be purer than 2E, and its higher N content indicates that this is so: The procedure followed in extracting 4E was suggested [Lugg, 1939], more particularly in association with fine maceration, but has not been used before.

Methods of estimation

Nitrogen estimations were made by the Kjeldahl method. Amide estimations were performed as described previously [Lugg, 1938, 3; Shore *et al.* 1936]. Tyrosine, diiodotyrosine and tryptophan were estimated by Lugg's [1937] methods (modified Millon colour reaction for tyrosine, special colour reaction between nitrous acid and tryptophan mercurial for tryptophan), following alkali and alkali-stannite hydrolyses of the protein preparations as described by Lugg [1938, 1].

RESULTS AND DISCUSSION

In Table 2 are shown in the order of the columns: the label of the protein preparation, its N content, and the amide, tyrosine and tryptophan contents as percentages of the protein-N appearing in these forms. The random errors, as percentages of the values shown, are believed not to exceed about 0.5, 1.5, 2.3 and 3.4 respectively.

Table 2. *Partial analyses of the proteins*

Prep.	% N	% amide-N	% tyrosine-N	% tryptophan-N
1W	3.92	5.52	2.43	1.70
2W	5.82	5.12	2.06	1.14
2E	11.10	4.97	2.08	1.13
2R	2.70	5.14	2.20	1.13
2E1	12.00	4.92	2.14	1.19
3W	3.12	5.31	2.60	1.43
4W	7.17	5.29	2.53	1.75
4E	14.10	5.35	2.64	1.62
4R	5.26	5.39	2.64	1.67
4'W	2.74	5.91	—	—

Table 3 gives a comparison of tyrosine and tryptophan estimations, following simple alkaline hydrolysis and alkali-stannite hydrolysis. Simple alkaline hydrolysis of these impure protein preparations normally does not provide as clean solutions as alkali-stannite hydrolysis does, and on this account the errors

Table 3. (Supplement to Table 2)

Prep.	Alkaline hydrolysis		Alkali-stannite hydrolysis	
	% tyrosine-N	% tryptophan-N	% tyrosine-N	% tryptophan-N
1W	2.36	1.65	2.46	1.72
2E 1	2.17	1.13	2.12	1.22
3W	2.53	1.47	2.65	1.40

of estimation tend to be higher in the former cases. Inasmuch as the former permits the estimation of tyrosine and the latter of tyrosine plus diiodotyrosine, the diiodotyrosine content follows by difference, though presumably any dibromotyrosine present would be included. Brand & Kassell [1939] have confirmed the procedure as satisfactory for the estimation of diiodotyrosine. The differences appearing in Table 3 are not considered significant, and the tyrosine and tryptophan contents shown in Table 2 are the weighted means of all estimations.

From the methods of extraction, preparations 2E, 2E1 and 4E were expected to be substantially representative of the whole proteins of the tissues, and the analyses, so far as they go, do not suggest that they were appreciably unrepresentative.

The leaf protein of *Trifolium subterraneum* does not depart, in these analyses, from the ranges of values obtained with the leaf proteins of other legumes. The protein of the petioles proved to be of higher amide content than that of the leaves.

The tyrosine and tryptophan contents of the *Pteridium aquilinum* preparations and the tryptophan content of the *Selaginella* sp. preparation are rather lower than those of the generality of angiosperm leaf proteins; in fact, the tryptophan contents of the *Pteridium aquilinum* preparations are lower than that of any of the angiosperm leaf proteins examined by these methods. However, it may be said that the amide, tyrosine and tryptophan contents of the proteins of the main photosynthesizing tissues of these cryptogams (*Lunularia cruciata* of the bryophytes, and *Pteridium aquilinum* and *Selaginella* sp. of the pteridophytes) are of much the same magnitude as those of the phanerogams examined [Lugg, 1938, 3; 1939; Chibnall, 1939].

It should be noted that the *Lunularia cruciata* preparation came from the gametophyte (with cell nuclei of n chromosomes), whereas the other cryptogam and all the phanerogam preparations came from sporophytes (with cell nuclei of $2n$ chromosomes).

SUMMARY

Partial analyses of protein preparations from the photosynthesizing tissues of one member of the bryophyte division (*Lunularia cruciata* of the class Hepaticae) and two members of the pteridophyte division (*Pteridium aquilinum* of the class Filicales and *Selaginella* sp. of the class Lycopodiales) have been made. The amide, tyrosine and tryptophan contents as percentage of the protein-N were respectively 5.52, 2.43 and 1.70 for *Lunularia cruciata*, 4.92-5.14, 2.06-2.20 and 1.13-1.19 for *Pteridium aquilinum*, and 5.31, 2.60 and 1.43 for *Selaginella* sp. These are of much the same magnitude as values obtained with leaf proteins of the angiosperms in the spermatophyte division, but the tryptophan contents of the *Pteridium aquilinum* preparations are rather lower than any encountered heretofore.

The amide, tyrosine and tryptophan contents of protein preparations from leaves of *Trifolium subterraneum* were 5.29-5.39, 2.53-2.64 and 1.62-1.75

respectively, as percentage of the protein-N (similar to other legumes), and the amide content of the petiole protein was 5.91.

Thanks are due to Mr R. A. Weller for valuable assistance, and to Prof. J. G. Wood, Miss B. S. Barrien and Miss C. M. Eardley for classification of plant material.

REFERENCES

- Bailey (1937). *Biochem. J.* **31**, 1406.
 Block (1934). *J. biol. Chem.* **105**, 455.
 — & Vickery (1931). *J. biol. Chem.* **93**, 113.
 Brand & Kassell (1939). *J. biol. Chem.* **131**, 489.
 Chibnall (1939). *Protein Metabolism in the Plant*. Yale Univ. Press.
 Lugg (1937). *Biochem. J.* **31**, 1422.
 — (1938, 1). *Biochem. J.* **32**, 775.
 — (1938, 2). *Biochem. J.* **32**, 2114.
 — (1938, 3). *Biochem. J.* **32**, 2123.
 — (1939). *Biochem. J.* **33**, 110.
 Shore, Wilson & Stueck (1936). *J. biol. Chem.* **112**, 407.