be regarded as representative of the whole-leaf proteins. Of the earlier preparations from *M. sativa* leaves, one by Osborne, Wakeman & Leavenworth (1921) contained 16.26 % N and one by Chibnall & Nolan (1924-5) contained 15.73 % N, but they were largely deficient in proteins associated with the chloroplasts and other granular protoplasmic bodies, and probably they both, more especially the former, contained degraded protein.

The degrees of extraction of the proteins were very good, in view of the coarse maceration technique used. The leaves of *M. denticulata*, being thin and pliant, are believed to have passed through the machine with less injury than the others, thus yielding less of their protein.

The partial compositions of the protein preparations are seen to be similar to those found for the W and representative E preparations from the leaves of other members of the Gramineae and Leguminosae. The impression gained earlier (Lugg, 1938 *b, c*) from work with preparations of uncertain representativeness, and supported by subsequent work (Lugg, 1939, 1943), that the whole-leaf proteins of the Leguminosae are probably, in general, of slightly smaller cyst(e)ine and methionine content than those of the Gramineae, has been further substantiated.

SUMMARY

1. The extraction of large samples of protein of reasonable purity from the leaves of four important

species of pasture plants has been described. The procedure, which was designed to secure reasonably representative extraction, involved the liberation (presumed) of protein from the chloroplasts and possibly other granular protoplasmic bodies, with the aid of a mildly alkaline (pH 9.2) buffer in the presence of lipid solvents (ethanol and ether). The differences in partial composition between the extracted proteins, and those in the original leaves and in the leaf residues, were so small and uncertain that the extracted proteins were considered to be reasonably representative of the whole proteins in the leaves.

2. The plant species were *Phalaris tuberosa*, *Hordeum murinum*, *Medicago sativa* and *Medicago denticulata*. The extracted protein preparations contained 14.90, 14.10, 14.15 and 14.55 % N, respectively. Of the N in these preparations there occurred respectively, 4.74, 5.04, 5.12 and 5.18 % as amide-N; 2.34, 2.31, 2.30 and 2.42 % as tyrosine-N; 2.08, 2.02, 1.94 and 1.87 % as tryptophan-N; 1.39, 1.34, 1.17 and 1.27 % as cyst(e)ine-N; and 1.57, 1.42, 1.36 and 1.32 % as methionine-N.

We wish to thank Prof. H. C. Trumble, Dr I. F. Phipps, Mr K. A. Pike and others of the staff of the Waite Agricultural Research Institute and Mr J. W. Walters for the leaf materials, and Prof. M. L. Mitchell for the use of the mincing machine. Thanks are due also to Mr D. S. Riceman and Miss C. M. Eardley for advice on agrostological and taxonomic questions.

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The Analysis of Hair Keratin

4. A NOTE ON THE OCCURRENCE OF SERINE IN HUMAN HAIR

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(Received 20 September 1944)

Vickery & Schmidt (1931) in their review make the statement that 'serine is one of the most difficult of all the amino-acids to isolate from proteins and is even yet an exceedingly rare substance'. A reagent

which has been used for precipitating *l*-serine from hydrolyzed silk fibroin, viz. *p*-hydroxy-azobenzene-*p*-sulphonic acid (Stein, Moore & Bergmann, 1941; Stein, Moore, Stamm, Chou & Bergmann, 1942),

may prove equally useful if applied to other protein hydrolysates, in which case the isolation of serine may become relatively easy.

Previous to the appearance of the publications of Stein *et al.*, a fraction of pure serine was obtained in a yield of 1.8% from a hydrolysate of human hair. It was impossible at that time to continue these studies. Subsequent work revealed that the quantity isolated formed only a small part of the total amount present, but since it was, so far as is known, the first time that serine had been obtained from human hair, the observation is reported here. Only small amounts of serine have been isolated from other keratins: sheep wool 0.1%, ram horn 1.1% (Abderhalden & Voitinovici, 1907); horse hair 0.6% (Abderhalden & Wells, 1905); goose feathers 0.4% (Abderhalden & Count, 1905); cattle horn 1.0% (Abderhalden & Heyns, 1932); wool keratin, over 2% (Martin & Synge, 1941).

EXPERIMENTAL

A portion of thoroughly washed human hair (equivalent to 112.6 g. ash- and moisture-free keratin) was refluxed for 11 hr. with 600 ml. 9N-HCl. Excess HCl was distilled off *in vacuo* and cystine was removed as the cuprous mercaptide (Lucas & Beveridge, 1940). After removal of copper from the combined filtrates with H_2S , the solution was concentrated *in vacuo* to about 350 ml. and some tyrosine which crystallized out was removed. The dicarboxylic acid fraction was precipitated with baryta and ethanol as already described (Beveridge & Lucas, 1944). After removal of inorganic ions the solution was concentrated *in vacuo*, a crop of leucine was taken off and the remaining amino-acids were converted to copper salts by repeated evaporation with copper carbonate (Town, 1928). The water-soluble, methanol-insoluble copper salts were freed from copper, the resulting solution containing 5.413 g. N. A sample containing 3.96 g. N was used for the subsequent steps. Histidine and arginine were taken out as the silver salts, lysine as the phosphotungstate, and from the filtrate all inorganic ions were removed. The resulting solution was evaporated to about 50 ml. and treated with 9 vol. of ethanol. A white, partly crystalline precipitate came down: wt. 2.272 g. (corrected for samplings, 3.11 g.); N, 12.9%. A portion (1.803 g.), recrystallized from 60% ethanol, yielded well-crystallized plates: wt. 1.147 g., corrected for sampling, 1.972 g.

The product did not exhibit optical activity, $[\alpha]_D^{21} = 0^\circ \pm 0.2$ (1N-HCl, $l=2$, $c=9.72$), presumably having been racemized during the many chemical procedures to which the hydrolysates had been subjected previously. The substance melted with decomposition at 243° (corrected); the accepted decomposition temperature of *dl*-serine (Fischer & Leuchs, 1902) is 246° (corrected).

Analytical data. Found: C, 34.6, 34.5; H, 6.76, 6.28; N, 13.3, 13.3%. Calc. for $C_3H_7O_3N$: C, 34.26; H, 6.72; N, 13.34%. A second recrystallization did not change the composition. The substance was undoubtedly *dl*-serine.

Further proof of the identity of this material was obtained by subjecting it to periodate oxidation (Nicolet & Shinn, 1941); the resulting formaldehyde was quantitatively isolated as the dimedon derivative (Vorlander, 1929). Synthetic *dl*-serine was subjected to the same procedure.

Dimedon-formaldehyde derivative from	Yield (%)	m.p. (corrected)
Isolated serine	96.6	190–191°
Synthetic serine	92.5	190–191°

Mixed m.p., 190–191°; quoted m.p., 189°.

Final proof that the fraction was indeed serine was obtained by subjecting it and a sample of authentic *dl*-serine to chloramine-T oxidation. The 2:4-dinitrophenylosazones of the resulting oxidation products were prepared as described by Damodaran & Ramachandran (1941).

2:4-Dinitrophenylosazone of glyoxal derived from	Yield (%)	m.p. (corrected)
Isolated serine	77.7	325°
Synthetic serine	73.1	324°

Mixed m.p., 324°; quoted m.p., 319°; m.p. of osazone from authentic glyoxal, 323°.

A determination performed later on a portion of the same sample of hair by the procedure of Nicolet & Shinn (1941) led to a value of 7.95%. This figure includes any formaldehyde derived from hydroxylysine (if present) and may therefore be an over-estimate of the actual serine content of human hair.

The work reported here is, as far as we can ascertain, the first isolation of serine from a protein hydrolysate by means of the copper salt method of fractionation.

SUMMARY

1. Serine has been isolated from a hydrolysate of human hair.

2. Application of the periodate oxidation procedure indicates that considerably more serine is present than was isolated.

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