# THE AMINO ACID COMPOSITION OF KERATINS

## I. THE AMINO ACID ANALYSIS OF MERINO 64'S QUALITY VIRGIN WOOL

By D. H. SIMMONDS*

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*Summary*

The ion exchange chromatographic technique of Moore and Stein (1951) has been used to estimate the amino acids in an acid hydrolysate of merino 64's quality virgin wool. A complete analysis of one sample of this wool is summarized in Table 3 together with figures for the carbon, hydrogen, nitrogen, sulphur, and ash contents. With the exception of the glycine analyses the results confirm the figures previously accepted as reliable for wool.

### I. INTRODUCTION

Although many estimations of individual amino acids in wool keratin have been made, no complete analysis of a single wool sample has yet been reported. Martin and Synge (1941*a*, 1941*b*, 1941*c*) and Gordon, Martin, and Synge (1943) were the first to evolve a method in which most of the neutral amino acids could be determined in the same sample of protein hydrolysate. This was achieved by counter-current extraction and by partition chromatography of the N-acetyl-amino acid derivatives. Previously, except for the work of Abderhalden and Voitinovici (1907), who used the ester distillation procedure of Fischer (1901), methods specific for individual amino acids had been used. The results of these early investigations are summarized in Table 1. For purposes of comparison all figures have been recalculated to amino acid nitrogen as percentage of total nitrogen, 16.5 per cent. being assumed to be the percentage total nitrogen in dry wool where no other data were given.

The present communication reports the complete amino acid analysis of a sample of 64's quality merino wool, as determined by the ion-exchange chromatographic method of Moore and Stein (1951), in conjunction with specific methods for cystine, methionine, tryptophan, and proline.

### II. EXPERIMENTAL AND RESULTS

#### (*a*) *Preparation of Wool for Analysis*

Samples of 64's quality virgin merino wool (5 g) were Soxhlet-extracted for 5 hr with 150 ml of 95 per cent. ethanol. The wool was squeezed, dried, and extracted with 200-ml portions of distilled water, until no further material absorbing light of wavelength 270-290 m$\mu$ was leached out. The wool was then air-dried, hand-picked to remove traces of seed and other impurities, and stored until required.

* Biochemistry Unit, Wool Textile Research Laboratory, C.S.I.R.O., Melbourne.

For the analyses, 3.0-g samples of extracted, air-dried wool from which discoloured tips had been removed were dried *in vacuo* over phosphorus pentoxide to constant weight. From these, three 0.05-g samples were weighed out and dried to constant weight in an oven at 110°C. Nitrogen was determined in the oven-dried samples by the micro-Kjeldahl technique. At the same time, 0.500 g (corrected for moisture content as determined above) was refluxed with 100 ml 6N HCl (twice glass-distilled) on an oil bath at 140-150°C for 16 hr. The HCl was removed by vacuum distillation and the concentrated solution was quantitatively transferred to a 50-ml measuring flask and made up to volume. For each analysis, 0.5 or 1 ml of this solution, as required, was measured into a small polythene cup and placed in an evacuated desiccator over $P_2O_5$ and KOH to remove water and HCl. Micro-Kjeldahl nitrogen determinations on the hydrolysate solution showed that manipulative losses were negligible.

### (*b*) *Preparation of Columns*

Finely ground Dowex 50 was wet-sieved through a 250-mesh sieve and treated as described by Moore and Stein (1951). The chromatographic columns were constructed from coarse porosity sintered glass funnels (SF8A, porosity 1 or 2) sealed to "Pyrex" tubing of 0.8-0.9 cm internal diameter drawn down to a rounded point as close to the sintered disc as possible. A B14 standard taper joint was sealed to the top of the column, which was surrounded by a jacket through which water could be circulated. The columns were graduated at 100 cm above the sintered glass plate for the separation of the acidic and neutral amino acids, and at 15 cm for the separation of the basic amino acids. A slurry of Dowex 50 in 0.2N NaOH was poured into the columns until, on settling under air pressure equivalent to 15 cm of mercury, the tube was filled with resin up to the graduation. To obtain columns having a uniform particle size gradient from top to bottom and giving amino acid bands of satisfactory sharpness, the resin bed was backwashed with 0.2N NaOH and allowed to settle with gentle tapping by hand or by rubber pressure tubing attached to the shaft of an electric motor. Columns were checked by means of a spirit level to make sure they were vertical before settling. Final adjustment to the graduation mark was made by pressurizing the column as required. When the 0.2N NaOH level had fallen to within 0.25 cm of the top of the resin, the appropriate buffer solution was carefully applied without disturbing the surface. The 100-cm columns were treated with 150-200 ml of pH $3.29 \pm 0.01$ citrate buffer, and the 15-cm columns with 50 ml of pH $5.00 \pm 0.01$ citrate buffer before use.

To regenerate the columns after a run they were treated with 0.2N NaOH until the effluent was alkaline. They were then backwashed, allowed to settle, and equilibrated with buffer as described above.

### (*c*) *Preparation of Buffer Solutions*

Except for omission of the detergent, the buffer solutions were prepared as described by Moore and Stein (1951) and stored at 5°C with the addition of thymol as a preservative. Just before use the appropriate quantity of buffer

TABLE 1

THE AMINO ACID COMPOSITION OF WOOL

| Reference* / Amino Acid | 1 | 2, 3 | 4 | 5, 6 | 7 | 8, 9, 10 | 11 | 12 | 13, 14, 15 | 16 | 17 | Miscellaneous Results |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alanine | 4·2 | | | | | | 3·9 | | 3·2 | | | |
| Arginine | | 18·4 | 15·2 | | 18·2 | | | 16·6 | 20·0 | 21·1 | 19·6 | 20·2 (18), 12·3 (19), 19·9 (20), 19·4 (21) |
| Aspartic acid | 1·5 | | | 4·6 | | | | | 3·8 | 4·7 | | 3·73 (22) |
| Amide N | | 6·6 | | 7·3 | | 7·2 | | | | | 7·6 | 8·6 (23), 7·2 (24), 8·7 (25) |
| Cystine | 5·1 | 8·5 | | | 9·9 | | | 8·5 | 8·0 | 9·9 | 5·6 | 8·0 (26), 6·1 (27), 7·4 (28), 9·0 (29), 8·4 (30), 6·9 (20), 8·3 (32), 8·3 (33), 8·9 (48), 8·4 (22), 7·9 (21), 9·2 (34) |
| Glutamic acid | 7·4 | | | 8·7 | | | | | 7·0 | 9·2 | | 9·3 (30) |
| Glycine | 0·7 | | | | 7·9 | | | | | | | |
| Histidine | | 10·5 | 1·1 | | 1·2 | | | | | 1·8 | 4·5 | 1·2 (19) |
| Hydroxylysine | | | | | | | | | | | | 0·11 (35), 0·11 (24) |
| Isoleucine | | | | | | 7·2 | | | | 3·0 | | |
| Isoleucine + Leucine | 7·4 | | | | | 7·4 | 6·2 | | 6·7 | | | |
| Leucine | | | | | | | | | | 5·3 | | |
| Lysine | | 3·02 | 2·7 | 3·8 | 3·1 | | | | | 3·9 | 7·7 | 2·5 (19), 3·8 (36) |
| Methionine | | | | | | 0·4 | 0·2 | | | 0·4 | | 0·25-0·38 (37), 0·34 (38), 0·11 (39), 0·32 (21), 0·35 (48) |
| Phenylalanine | | | | | 2·2 | 1·9 | 0·8 | | 1·9 | 2·1 | | |
| Proline | 3·2 | | | | | 4·9 | 5·2 | | 3·8 | 6·1 | | |
| Serine | 0·08 | | | | | 7·5 | | 7·6 | 12·8 | | | 8·2 (23), 7·7 (26) |
| Threonine | | | | | | 4·7 | | | | 4·8 | | 4·6 (23) |
| Tryptophan | | 1·4 | | | 0·6 | | | | | | | |
| Tyrosine | 1·4 | 2·1 | | | 2·3 | | 2·8 | 2·8 | 2·6 | 2·7 | | 2·4 (40), 2·7 (41), 2·7 (42) |
| Valine | 2·0 | | | | | 3·4 | 3·9 | | 3·6 | 4·2 | | |
| Total estimated | 32·6 | 40·0† | 19·0 | 24·4 | 45·4 | 37·2 | 23·0 | 35·5 | 73·4 | 79·2 | 44·9 | |
| Total N | | 17·6–18·0 | 16·57 | | 15·4 | | | 16·67 | 16·9 | 16·2 | 16·14 | 16·50-17·07 (43), 16·4 (30), 16·57 (24), 16·43 (19), 16·3 (48), 16·3 (23), 16·29 (32), 16·66 (33), 16·50 (28) |
| Total S | | 3·52–3·59 | 4·52 | | 3·6 | | | 3·5 | 3·76 | | 2·91 | 2·96-3·97 (37), 3·34 (32), 3·19 (33), 3·47 (28), 3·39-3·73 (44), 3·64-3·35 (45), 3·58 (40), 3·63 (48), 3·35 (39), 3·43 (34), 3·03 (46), 3·50 (26) |
| Mean residue weight | 118 (31), 116, 107 (47) | | | | | | | | | | | |

was boiled to expel air, placed in a separating funnel, and covered with a layer of paraffin oil. To achieve satisfactory separation of glycine, alanine, cystine, and valine, it was found necessary to adjust the pH of the citrate buffer to 3.29, instead of 3.42 as specified by Moore and Stein (1951). The optimum value for this buffer can only be determined by trial and error in successive runs.

The ninhydrin reagent was prepared according to the directions of Moore and Stein (1948) but the solution used for diluting the colour developed after heating was a 50 per cent. (v/v) ethanol-water mixture in place of the *n*-propanol mixture recommended by them. The ninhydrin reagent and the diluent were both dispensed by pipettes each consisting of a hypodermic syringe connected through a three-way "Pyrex" stopcock to a reservoir on one side and a constricted outlet on the other. One bore of the three-way tap was blocked to prevent liquid passing directly from the reservoir to the outlet. The pipettes were calibrated by discharging them the requisite number of times into a graduated flask. This procedure was followed each day to draw off reagent solution which had been standing in contact with the polyvinyl chloride tubing connecting the pipette to the reservoir, otherwise the first three or four tubes examined were slightly turbid.

### (*d*) *Measurement of Colour Yields and Calibration of the Spectrophotometer*

The colour yields for seven of the amino acids given by Moore and Stein (1948, 1951) were checked by dividing the regression coefficient relating their concentration with optical density at 570 m$\mu$, by the corresponding coefficient for leucine. These amino acids were purified to give correct analytical figures for carbon, hydrogen, and nitrogen, and they yielded single spots when examined by paper chromatography. Standard solutions in pH 5.0 citrate buffer were prepared containing 14 dilutions of each amino acid between 0.0005 and 0.06 mg of amino nitrogen per ml, and the colour was developed in 1-ml aliquots of these solutions as described by Moore and Stein (1948). The colour density was read in a Uvispek spectrophotometer against a blank solution containing buffer, reagent, and diluent. Where the optical density for a 1-cm light path was greater than 1.000, 1 ml of the solution was mixed with 5 ml of diluent

* 1. Abderhalden and Voitinovici (1907). 2. Marston (1928). 3. Marston (1932). 4. Vickery and Block (1930). 5. Asquith and Speakman (1952). 6. Speakman and Townend (1937). 7. Block (1939). 8. Martin and Synge (1941*a*). 9. Martin and Synge (1941*b*). 10. Martin and Synge (1941*c*). 11. Gordon, Martin, and Synge (1943). 12. Geiger (1944). 13. Lindley and Phillips (1945). 14. Lindley (1947). 15. Lindley and Drucker (personal communication). 16. Graham, Waitkoff, and Hier (1949). 17. Stakheyewa-Kaverznewa and Gavrilow (1937). 18. Vickery (1940). 19. Stewart and Rimington (1931). 20. Graff, Macula, and Graff (1937). 21. Cuthbertson and Phillips (1945). 22. Bailey (1942). 23. Rees (1946). 24. Chibnall (1942). 25. Steinhardt (1942). 26. Mizell and Harris (1943). 27. Vickery and White (1933). 28. Geiger and Harris (1942). 29. McCallum (1942). 30. Olcott (1944). 31. Astbury (1942). 32. Goddard and Michaelis (1935). 33. Goddard and Michaelis (1934). 34. Rimington (1929*a*). 35. van Slyke, Hiller, and MacFadyen (1941). 36. Rutherford, Harris, and Smith (1937). 37. Barritt (1934*a*). 38. Blackburn, Carter, and Phillips (1941). 39. Mueller (1923). 40. Elliott and Speakman (1943). 41. Harris, Mizell, and Fourt (1942). 42. Steinhardt and Harris (1940). 43. Barritt (1928). 44. Barritt (1934*b*). 45. Barritt (1927). 46. Rimington (1929*b*). 47. Tristram (1949). 48. Bailey (1937).

† Histidine figure not included.

and read against the blank similarly diluted. Table 2 shows the regression coefficients thus obtained and the ratio of these to that of leucine. The ratios agree closely with those of Moore and Stein (1948, 1951) except for glutamic acid and glycine. In subsequent work a duplicate series of standard leucine solutions was included with each analytical run. The regression coefficient calculated from these data was used to convert all the optical density readings into mg of amino nitrogen.

TABLE 2

COLOUR YIELDS OF AMINO ACIDS

| Amino Acid | Concn. Range (mg N/ml) ($\times 10^4$) | Regression Coefficient and Standard Error | Colour Yield (Leucine = 1·00) |
|---|---|---|---|
| Alanine | 7·9—79 | 193·9±1·9 | 1·00 |
| Aspartic acid | 5·3—84 | 185·6±0·68 | 0·95 |
| | 63—530 | 29·33±0·35 | 0·93 |
| Cystine | 6·0—119 | 105·4±0·38 | 0·54 |
| Glutamic acid | 4·7—75 | 189·4±2·3 | 0·97 |
| | 57—570 | 31·16±0·16 | 0·99 |
| Glycine | 9·4—94 | 188·8±3·8 | 0·97 |
| | 56—560 | 30·71±0·20 | 0·98 |
| Leucine | 5·3—63 | 194·5±1·4 | 1·00 |
| | 63—630 | 31·51±0·15 | 1·00 |
| Valine | 5·9—59 | 191·4±2·8 | 0·98 |
| | 71—589 | 31·57±0·13 | 1·00 |

### (*e*) *Apparatus and Analytical Procedure*

The Dowex 50 columns were set up over a solenoid-operated fraction collector so that the effluent passed through a platinum wire grid before falling into a test-tube (15.4 × 1.8 cm). The momentary passage of an electric current across the grid operated an electronic counter. After the delivery of a pre-set number of drops, the solenoid moved the collector turntable forward one position.

Difficulty was experienced in maintaining the fraction size constant as the temperature of the columns was varied between 37.5 ± 0.5°C and 75 ± 1°C. Compensation could usually be made by increasing the number of drops per fraction each time the temperature was raised, so that the weight of liquid obtained, after adjustment of the pH to 5.0 for the development of colour with ninhydrin, was 1.00 ± 0.05 g. Where this could not be done, and the fractions were smaller than 0.95 g, the necessary amount of buffer was added to bring each fraction up to 1.00 g. In later experiments a cooling tube was introduced between the column tip and the conductivity grid, but this only partly resolved the difficulty. Further work has improved the accuracy of fraction measurement, and the apparatus used will be described in a forthcoming publication.

About 1 hr before use water at 37.5 ± 0.5°C (25°C for the 15-cm columns) was circulated through the column jackets and the level of the buffer above the resin was allowed to fall to within 5 mm of the resin surface. The residue obtained by evaporating 0.5 ml of the wool hydrolysate, was dissolved in 0.5 ml of pH 3.29 buffer and transferred to the top of the column. It was allowed to sink in under atmospheric pressure and three or four washings, each of 0.3 ml buffer, were then used to rinse the polythene cup and sides of the column. Finally, the column was filled to the top with buffer and connected by means of polyvinyl chloride tubing with the buffer reservoir which could be raised or lowered to control the rate of flow of the column.

The procedure followed during an analytical run was similar to that described by Moore and Stein (1951) and is summarized, together with the results of a typical analysis, in Figures 1 and 2, where the colour density at 570 mμ of each tube corrected for colour yield, is plotted against fraction number.

The identity of each amino acid peak in the figures (cf. Moore and Stein 1951) has been confirmed by paper chromatography of appropriate fractions taken from the effluent of the Dowex 50 columns. After desalting (Simmonds 1953*b*), each fraction was chromatographed with and without the appropriate amino acid as a marker. In each case only one spot was observed on the paper chromatograms after spraying with ninhydrin. The only peaks of doubtful identity are those labelled unknown (1) and unknown (2) in Figure 1.

### (*f*) *Estimation of Other Amino Acids*

(i) *Cystine.*—Since the colour yield of cystine is low (Moore and Stein 1948) and it tends to emerge from the column as a broad plateau it was estimated in a separate sample of the same wool by the method of Shinohara (1935, 1937). The results of these analyses are included in Table 3.

(ii) *Methionine.*—As thiodiglycol was not available as an anti-oxidant, methionine was determined by the volatile iodide method of Baernstein (1932, 1936).

(iii) *Tryptophan.*—This amino acid was estimated spectrophotometrically by the method of Goodwin and Morton (1946). A sample of wool (30 mg) was dissolved by warming for 10 min in 10 ml 1N NaOH. The clear solution was cooled and diluted to 100 ml with water. Solutions containing 10 ml 1N NaOH alone and together with 10 mg tryptophan were treated similarly and no loss of tryptophan was observed in the latter. The samples were read in a Beckmann spectrophotometer at 280, 294.4, 340, and 370 mμ, using 0.1N NaOH as a blank.

(iv) *Proline.*—Contrary to the experience of Moore and Stein (1951) glutamic acid and proline were not resolved sufficiently in the Dowex 50 column effluents to permit accurate estimation of the latter with ninhydrin at pH 5.0. Proline was therefore estimated by conducting the ninhydrin reaction at pH 1 in the presence of phosphoric and acetic acids (Chinard 1952). The reagent was freshly prepared as required, by dissolving 2.5 g of ninhydrin in a mixture of 40 ml 6M $H_3PO_4$ and 160 ml glacial acetic acid. Aliquots (2 ml) of the

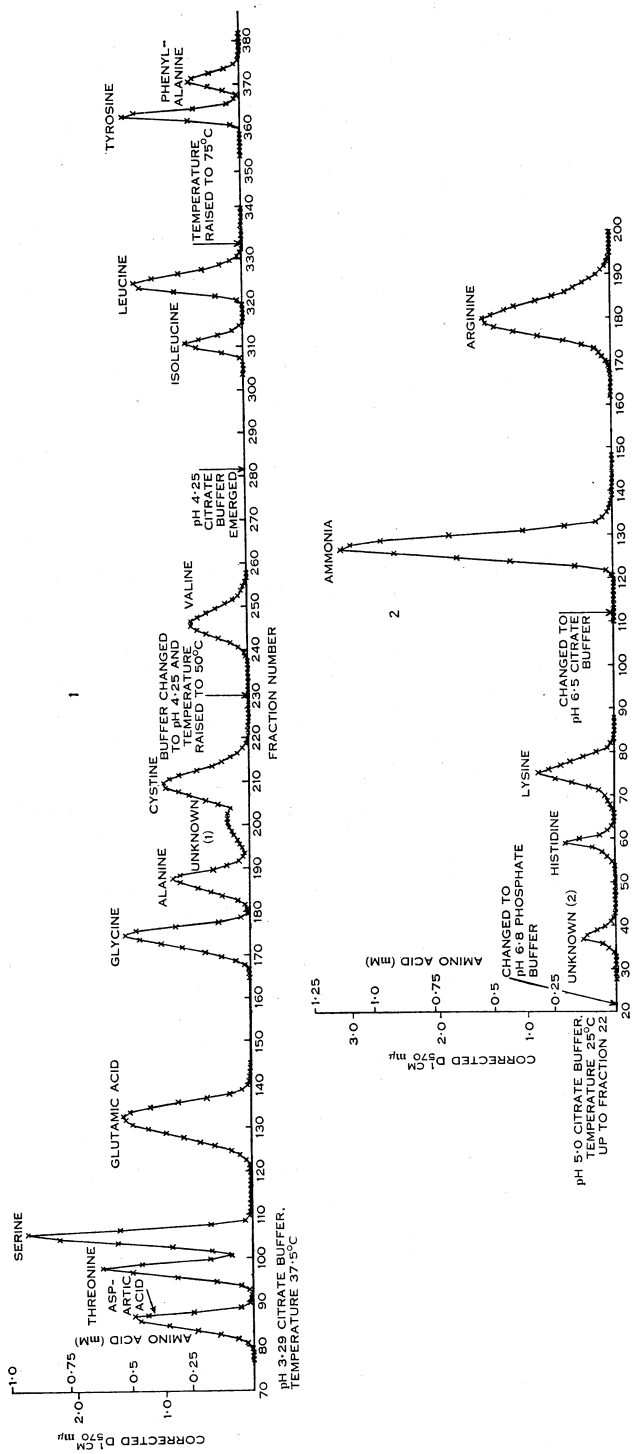


Fig. 1.—Separation on a 100-cm Dowex 50 column of acidic and neutral amino acids in hydrolysate of 64's quality merino wool. Column loading 0.750 mg N.

Fig. 2.—Separation on a 15-cm Dowex 50 column of basic amino acids in hydrolysate of 64's quality merino wool. Column loading 1.658 mg N.

proline-containing solutions were adjusted with 2N NaOH to the first pink colour of phenolphthalein, and then with 0.1N HCl until the indicator was just colourless. Sodium nitrite (6M, 2 ml) and glacial acetic acid (1 ml) were added and the deamination was allowed to proceed according to the method of Troll (1953). After evaporating to a small volume three times with 5 ml conc. HCl, the residue was neutralized as before with 2N NaOH and 0.1N HCl, 5 ml 6M $H_3PO_4$ and 5 ml glacial acetic acid were added, and the solution was diluted to 50 ml with distilled water. Aliquots of this solution (0.5, 1.0, 1.5, and 2.0 ml) were withdrawn for analysis and made up with a deaminated mixture of 14 amino acids (excluding proline) to 2.0 ml total volume. To each aliquot, reagent (4 ml), was added and the solutions were heated in a boiling water-bath for 30 min—the optimum time for colour development. The tubes were diluted with glacial acetic acid (4 ml) and were read in a Uvispek spectrophotometer at 515 m$\mu$.

Samples of the original wool hydrolysate, a standard solution containing 0.70 mg of proline per ml, and a reagent blank consisting of a mixture of 14 amino acids (excluding proline) in the proportions in which they occur in wool, were taken through the procedure outlined above.

### (*g*) *Calculation of Results*

(i) *Amino Acids in the Effluent of the Dowex* 50 *Columns.*—The optical density readings obtained for each amino acid were summed, converted into leucine equivalents by multiplying by the reciprocal of the colour yield, and divided by the regression coefficient obtained from the standard leucine control series. The results were expressed as a percentage of the nitrogen determined on an aliquot of the hydrolysate by the micro-Kjeldahl method.

(ii) *Tyrosine and Tryptophan.*—The total quantity of tyrosine and tryptophan present was calculated from the optical density reading at 294.4 m$\mu$, the point of intersection of the absorption curves of these two amino acids. From the reading at 280 m$\mu$ the amount of each amino acid was obtained using the equation given by Goodwin and Morton (1946).

(iii) *Proline.*—Calculation of the percentage of proline nitrogen present was carried out by the slope ratio assay method (Clarke 1952).

### (*h*) *Results*

Table 3 summarizes the results obtained in the present investigation, together with the standard error associated with the mean in each case.

## III. Discussion

The wool fibre consists of at least three distinct morphological components (Lindberg *et al.* 1949) and is therefore almost certainly not a homogeneous protein. Chemical (Goddard and Michaelis 1935; Alexander 1951; Horio and Kondo 1953; Mercer 1953; Fraser and Rogers 1953), electrophoretic (Gillespie

TABLE 3

AMINO ACID COMPOSITION OF MERINO 64'S QUALITY WOOL

Carbon (dry weight) 50·23%; hydrogen 8·13%; nitrogen 16·62%; sulphur (dry weight) 3·68%; ash 0·01%; moisture 11·55%

| Amino Acid | No. of Analyses | Mean of Total Nitrogen and Standard Error (%) | Weight from 100 g Dry Wool | Weight Residues in 100 g Dry Wool | G-equiv./ $10^4$ g Dry Wool |
|---|---|---|---|---|---|
| Alanine | 7 | 3·51±0·08 | 3·71 | 2·96 | 4·17 |
| Arginine | 4 | 20·32±0·04 | 10·49 | 9·40 | 6·02 |
| Aspartic acid | 8 | 4·24±0·18 | 6·69 | 5·78 | 5·03 |
| Amide N | 4 | 7·46±0·57** | 1·42 | 1·42 | 8·86 |
| Cystine | 11 (5) | 7·93*(4·95)±0·05 (±0·19) | 11·30 | 10·45 | 4·71 |
| Glutamic acid | 7 | 8·58±0·13 | 14·98 | 13·15 | 10·18 |
| Glycine | 7 | 5·80±0·09 | 5·16 | 3·92 | 6·88 |
| Histidine | 5 | 1·46±0·10 | 0·90 | 0·80 | 0·57 |
| Isoleucine | 8 | 1·97±0·05 | 3·07 | 2·65 | 2·34 |
| Leucine | 8 | 4·90±0·12 | 7·63 | 6·58 | 5·82 |
| Lysine | 5 | 3·25±0·15 | 2·82 | 2·47 | 1·93 |
| Methionine | 2 | 0·39‡±0·02 | 0·69 | 0·61 | 0·46 |
| Phenylalanine | 6 | 1·75±0·09 | 3·43 | 3·06 | 2·08 |
| Proline | 2 | 5·33§±0·05 | 7·28 | 6·14 | 6·33 |
| Serine | 8 | 7·25±0·19 | 9·04 | 7·50 | 8·61 |
| Threonine | 8 | 4·61±0·13 | 6·55 | 5·56 | 5·47 |
| Tryptophan | 4 | 1·73‖±0·12 | 2·10 | 1·91 | 1·03 |
| Tyrosine | 5 | 2·97 (3·08‖)±0·08 | 6·38 | 5·75 | 3·53 |
| Unknown (1) | 5 | 1·18 (2·22¶)±0·10 | — | — | — |
| Unknown (2) | 4 | 0·71†±0·07 | 0·68 | 0·60 | 0·42 |
| Valine | 7 | 3·57±0·10 | 4·96 | 4·20 | 4·24 |

* Estimated by method of Shinohara (1935, 1937).

† Assumed to be hydroxylysine with a colour yield the same as lysine.

‡ Estimated by method of Baernstein (1932, 1936).

§ Estimated by method of Chinard (1952).

‖ Estimated by method of Goodwin and Morton (1946).

¶ Corrected for colour yield of cystine (see discussion).

** Uncorrected for decomposition of serine and threonine during hydrolysis.

and Lennox 1953), molecular weight (Ward 1952; Friend and O'Donnell 1953), and end-group studies (Middlebrook 1951) further emphasize its heterogeneity. Amino acid analyses such as those presented above are mainly of value therefore in permitting a comparison between the overall composition of different types of wool. However, it may be pertinent to discuss the similarities and differences observed between the results obtained in this and previous investigations.

In general the results confirm those regarded as reliable in the literature (references 5-15, Table 1) but they are somewhat lower than those reported by Graham, Waitkoff, and Hier (1949) (reference 16, Table 1). The major differences lie in the values reported for glycine, and the ratio of leucine to isoleucine (Simmonds 1953*a*). The only analysis for glycine previously reported (7.9 per cent. nitrogen (Block 1939)) is substantially higher than the figure of 5.8 per cent. recorded in the present investigation.

Speakman and co-workers (Speakman and Townend 1937; Asquith and Speakman 1952) have carried out analyses for the acidic and basic amino acids of wool to determine whether the sum of the free carboxyl groups of glutamic and aspartic acids is equivalent to that of the free basic groups of arginine, histidine, and lysine. The data in Table 3 support the conclusions of Speakman and co-workers in indicating that this is approximately so, provided that tyrosine is included with the acidic amino acids (cf. Steinhardt and Harris 1940). The figures below are expressed as gram-equivalents of free acidic and basic groups in $10^4$ g of wool.

| Free Carboxyl Groups | | Free Basic Groups | |
|---|---|---|---|
| Glutamic acid | 10.18 | Unknown (2) (see footnote to Table 3) | 0.71 |
| Aspartic acid | 5.03 | Lysine | 1.93 |
| Tyrosine — OH | 3.53 | Arginine | 6.02 |
| | | Histidine | 0.57 |
| | | Amide | 8.86 |
| | 18.74 | | 18.09 |

The results shown in Table 3 indicate that the amount of nitrogen unaccounted for in the present investigation is small. The only two peaks of doubtful identity, labelled unknown (1) and unknown (2) in Figure 1, are being currently investigated.

The only element which has not been completely accounted for is sulphur. The figures for cystine sulphur (3.02 per cent. dry weight) and methionine sulphur (0.15 per cent. dry weight) fall short of the total sulphur estimated (3.68 per cent.) by 0.51 per cent. A similar discrepancy in the sulphur analyses has been reported by Barritt (1934*a*), Bailey (1937), Schöberl and Rambacher (1940), Schöberl (1952), Cuthbertson and Phillips (1945), and Marston (1946). In the present work, part of the missing sulphur was present as cysteic acid and the peak corresponding to this constituent was observed in the effluent of the Dowex 50 columns preceding the emergence of aspartic acid. However,

there was insufficient cysteic acid or any other unidentified component to account for the remaining sulphur as a sulphur-containing amino acid. The unidentified sulphur is also unlikely to be present as sulphate since the ash content of the wool is low and the method used for the determination of total S (Grote and Krekeler 1933) does not estimate sulphur in the form of sulphate.

A further discrepancy arises when the percentage of cystine calculated by the Shinohara (1935, 1937) and the Moore and Stein (1951) procedures are compared (see Table 3). Because of the small peak of unknown (1) preceding it, and the sometimes irregular nature of the cystine peak, it was difficult to estimate the latter as accurately as the other amino acids. A low recovery of cystine has similarly been observed by Stein (1953). The Shinohara technique may therefore estimate reducing material other than cysteine, or the cystine may be racemized or destroyed during the 16 hr hydrolysis to which the wool sample is subjected before Moore and Stein analysis. Toennies and Bennett (1935) have discussed the extent of racemization of cystine occurring during acid hydrolysis, and since the DL- and L- forms differ considerably in solubility it is possible that unknown (1) is one stereoisomeric form of cystine partially separated from the other. If this were so, the total cystine content would be 7.17 per cent., which is in closer agreement with the figure obtained by the Shinohara method. When a synthetic mixture of amino acids, simulating the composition of wool but not subjected to acid hydrolysis, was examined by the Moore and Stein procedure, the cystine peak was observed to be perfectly regular and it accounted quantitatively for the cystine known to be present.

## IV. Acknowledgments

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