

# THE AMINO ACID COMPOSITION OF KERATINS

## II. THE AMINO ACID COMPOSITION OF A KERATIN DERIVATIVE EXTRACTED FROM WOOL WITH ALKALINE THIOGLYCOLLATE SOLUTION

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### Summary

The amino acid composition of a protein component of Merino 64's quality wool, which moves as a single peak on electrophoresis in alkaline thioglycollate solutions, has been determined. The results, which are summarized in Table I, show that the purified protein fraction contains more aspartic acid, glutamic acid, leucine, lysine, and amide nitrogen, and less cystine, proline, serine, and tryptophan, than the parent wool from which it was extracted.

### I. INTRODUCTION

Gillespie and Lennox (1953, 1955) have demonstrated the presence of several protein constituents in dispersions of wool in alkaline potassium thioglycollate solutions. They have described the extraction of a protein constituting the main component of the dispersion, which moves as a single peak on electrophoresis in alkaline buffers containing potassium thioglycollate.

In the present paper, preliminary data on the amino acid composition of this protein are presented, and the results are compared with a similar amino acid analysis of whole Merino 64's quality wool (Simmonds 1954*a*).

### II. EXPERIMENTAL

#### (a) Preparation of the Sample for Analysis

The protein preparation was extracted from Merino 64's virgin wool or dry combed top according to the procedure of Gillespie and Lennox (1955). The fraction containing the main component (fraction "F" of Gillespie and Lennox 1955), was checked to ensure the presence of only a single peak on electrophoresis at pH 11.0 under the conditions described by them. The pH of this fraction was then adjusted to 5.0 to precipitate the protein, and the precipitate dissolved in one-tenth the original volume of 0.1M potassium thioglycollate at pH 10.5 and dialysed against running tap water. The solution was freeze-dried and the protein obtained as a fluffy white powder, which was difficult to handle because of its electrostatic charge.

Two samples of approximately 50 mg were placed in test tubes containing 10 ml of twice-distilled constant-boiling HCl. The tubes were evacuated and

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sealed. Hydrolysis at 110°C was carried out for 22 and 70 hr respectively, at the end of which time the tubes were opened and the contents evaporated *in vacuo*. Each was made up to 10 ml with water, and the nitrogen content determined by the micro-Kjeldahl technique. Samples of each hydrolysate were then pipetted into polythene cups and evaporated over KOH and P<sub>2</sub>O<sub>5</sub> to remove HCl before application to the "Dowex 50" columns. The sample size was adjusted so that the loading was approximately 0.3 mg nitrogen on the 100-cm "Dowex 50" columns, and 0.6 mg nitrogen on the 15-cm "Dowex 50" columns.

Cystine was determined by oxidation to cysteic acid using performic acid, according to the method of Schram, Moore, and Bigwood (1954). The oxidized and hydrolysed samples, after evaporation nearly to dryness twice with distilled water in a rotary evaporator of the type described by Craig, Gregory, and Hausmann (1950), were made up to 10 ml with distilled water. After determination of the nitrogen content, samples were placed in polythene cups and evaporated to dryness over KOH and P<sub>2</sub>O<sub>5</sub>.

#### (b) Analytical Procedure

The ion exchange chromatographic and ninhydrin colorimetric procedures of Moore and Stein (1948, 1951) were used to estimate individual amino acids. Preparation of the columns and solutions and conduct of the analyses were as previously described (Simmonds 1954a), except that a magnetic balance (Simmonds 1954b) was used for the collection of 1-ml fractions.

Tryptophan and proline were determined by methods previously described (Simmonds 1954a). Cysteic acid was separated from the other amino acids in the oxidized protein samples on a 100-cm "Dowex 50" column equilibrated with pH 3.30 citrate buffer. A preliminary run on the unoxidized protein component showed complete absence of ninhydrin-positive material before the emergence of aspartic acid. With the oxidized samples cysteic acid emerged unretarded as a symmetrical peak at the buffer front (about tube 35). Two samples of purified cystine treated in a manner identical with that of the protein component gave an average overall recovery of  $96.47 \pm 0.12$  per cent., and the cystine figure obtained from the protein hydrolysate was accordingly corrected for decomposition during oxidation and hydrolysis by this factor, as suggested by Schram, Moore, and Bigwood (1954). There is some evidence, however (Thompson, personal communication), that small changes in the conditions of oxidation and hydrolysis affect the recovery of certain amino acids, and the cystine figure quoted in Table 1 is to be regarded as tentative.

We have been unable to estimate the proportion of methionine present in the preparation since the performic acid oxidation product, methionine sulphone, appears at about tube 110, incompletely separated from serine. Because of the small amount of methionine present in the protein, we were unable to detect it in the unoxidized samples, and so far insufficient material has been available to allow us to use the conventional Baernstein procedure (Baernstein 1932, 1936).

## III. RESULTS

Elution curves for 22-hr hydrolysates of oxidized and unoxidized protein samples are compared in Figure 1, while Table 1 summarizes the results of these analyses together with data derived from 70-hr hydrolysates of unoxidized material. Figures for the tryptophan, proline, nitrogen, and sulphur contents are also included.

TABLE 1

AMINO ACID COMPOSITION OF HYDROLYSATES OF A KERATIN DERIVATIVE EXTRACTED FROM MERINO 64's QUALITY WOOL

Amino acid nitrogen expressed as a percentage of total nitrogen. Nitrogen:  $15.26 \pm 0.05$  per cent. Sulphur: 2.55 per cent.

Treatment	Unoxidized Samples						Oxidized Samples			Averaged or Adjusted Results. Data of Columns 2 and 3
	22-Hr Hydrolysate			70-Hr Hydrolysate			22-Hr Hydrolysate			
Amino Acid	No. of Analyses	Mean	S.E.	No. of Analyses	Mean	S.E.	No. of Analyses	Mean	S.E.	
1		2			3			4		5
Alanine	3	4.22	0.22	3	4.03	0.22	2	4.62	0.27	4.13
Arginine	2	21.12	0.40	3	19.87	0.32	2	18.40	0.40	20.50
Aspartic Amide N	4	5.68	0.34	3	5.09	0.40	2	5.70	0.48	5.39
Cystine	3	11.56	0.09	3	11.54	0.09	2	12.92	0.11	11.56
Glutamic	3	10.82†	0.09	3	9.10†	0.09	2	4.33	0.03	4.33*
Glycine	3	10.76†	0.11	3	9.10†	0.09	2	10.76†	0.11	11.72
Histidine	3	5.27	0.28	3	4.87	0.28	2	5.70	0.34	5.07
Isoleucine	2	1.42	0.07	3	1.32	0.06	2	0.24	0.07	1.37
Leucine	4	2.24	0.10	3	2.25	0.11	2	2.38	0.14	2.25
Lysine	4	6.01	0.14	3	5.95	0.17	1	5.39	0.29	5.98
Phenylalanine	3	5.03	0.14	3	4.46	0.14	2	4.46	0.17	4.65
Proline	4	1.72	0.13	3	1.68	0.15	—	—	—	1.70
Serine	1	3.66								3.66
Threonine	4	6.47	0.08	3	6.12	0.09	2	5.98	0.11	6.66
Tryptophan‡	3	4.44	0.13	3	3.34	0.13	2	3.54	0.16	5.05
Tyrosine	4									0.71‡
Valine	3	2.46	0.12	3	2.10	0.13	—	—	—	±0.03
	3	3.55	0.18	3	3.91	0.18	2	3.86	0.21	2.28
										3.74

\* From data of column 4.

† Corrected for pyrrolidone carboxylic acid formation (Moore and Stein 1951).

‡ Method of Goodwin and Morton (1946).

Data derived from the figures in column 5 of Table 1 are summarized in Table 2. This shows the weight of each amino acid and amino acid residue obtainable from 100 g of the protein, together with data on the number of

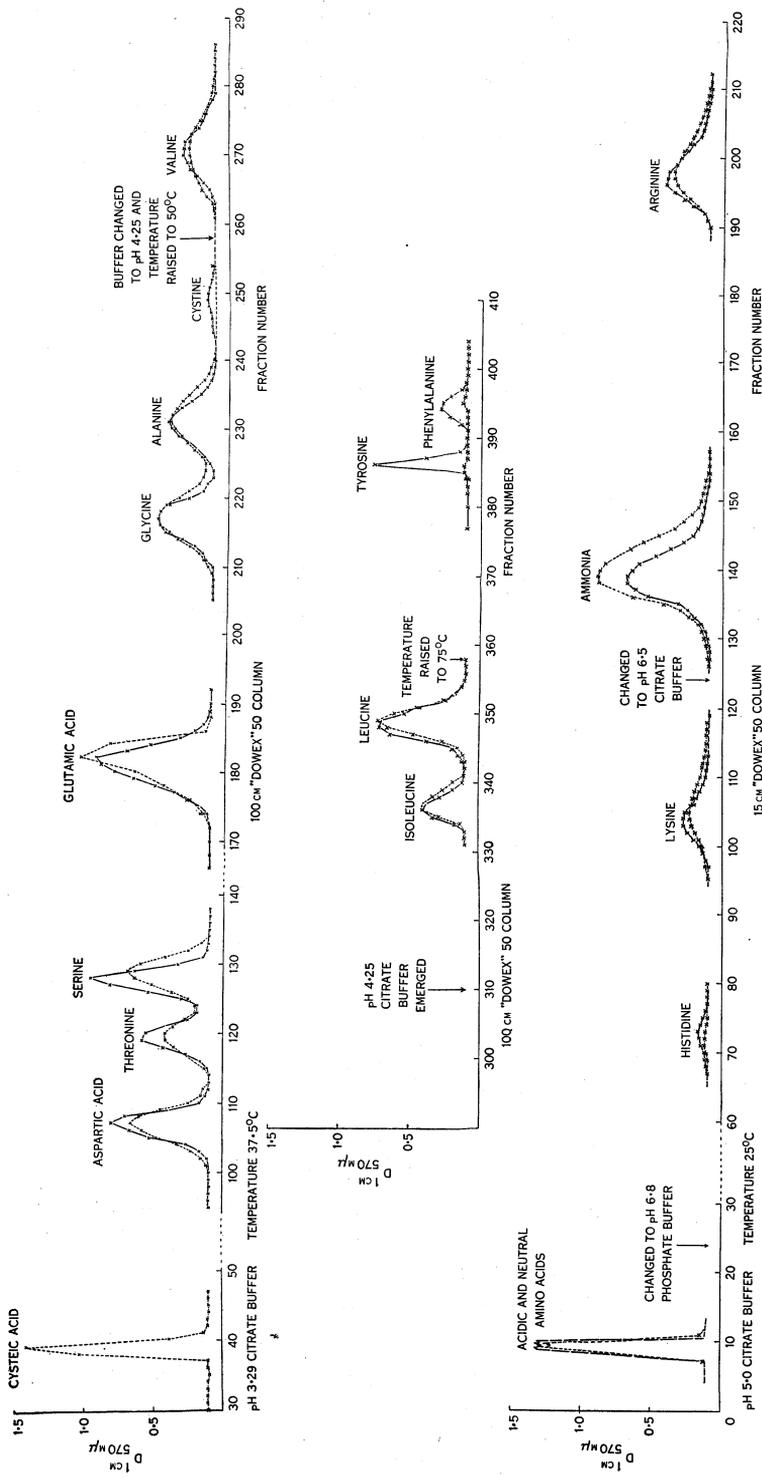


Fig. 1.—Comparison of 22hr hydrolysates of a keratin derivative. ×———×, Unoxidized. ×-----×, After oxidation with performic acid by the method of Schram, Moore and Bigwood (1954).

residues of each amino acid contained in an assumed molecular weight of 15,000, which is probably close to the true value as indicated by the studies of Harrap (1955). The figures used in the calculations for serine, threonine, and glutamic acid were obtained by extrapolation to zero time of hydrolysis as suggested by Smith and Stockell (1954).

TABLE 2  
AMINO ACID COMPOSITION OF A KERATIN DERIVATIVE EXTRACTED FROM MERINO 64's QUALITY WOOL

Derived from data in column 5 of Table 1

Amino Acid	Wt./100 g Dry Protein	Wt. Residues/100 g	No. of Residues per M.W. 15,000	
Alanine	4.01	3.20	6.75 $\approx$	7
Arginine	9.72	8.71	8.19	8
Aspartic	7.81	6.75	8.81	9
Amide	2.02	2.02	18.90	19
$\frac{1}{2}$ Cystine	5.67	4.82	14.16	14
Glutamic	18.79	16.49	19.16	19
Glycine	4.14	3.15	8.29	8
Histidine	0.77	0.68	0.75	1
Isoleucine	3.21	2.77	3.68	4
Leucine	8.54	7.37	9.78	10
Lysine	3.70	3.24	3.80	4
Phenylalanine	3.86	3.44	2.78	3
Proline	4.59	3.87	5.98	6
Serine	7.62	6.33	10.89	11
Threonine	6.59	5.59	8.26	8
Tryptophan	0.79	0.72	0.58	1
Tyrosine	4.50	4.05	3.73	4
Valine	4.76	4.03	6.10	6

#### IV. DISCUSSION

In view of recent reports of the destruction of amino acids during acid hydrolysis (Hirs 1954; Smith and Stockell 1954; Smith, Stockell, and Kimmel 1954) we have prepared and analysed one 22-hr and one 70-hr hydrolysate of the purified wool protein fraction, together with 22-hr hydrolysates of two samples which had been subjected to performic acid oxidation. An analysis of the data in Table 1 has shown that in the 22-hr and 70-hr hydrolysates of the unoxidized protein, the glutamic acid figures differ significantly at the 1 per cent. level, while those for serine and threonine differ at the 5 per cent. level. The remainder, including those for aspartic acid and arginine, are not significantly different at the 5 per cent. level. Only the figures for glutamic acid, serine, and threonine have therefore been linearly extrapolated to zero time of hydrolysis, while the remaining figures have been averaged. These results are recorded in the final column of Table 1. A puzzling feature of the results is that there is no corresponding increase from 22 to 70 hr, in the figures for am-

monia nitrogen, as has been reported by Hirs (1954), Smith and Stockell (1954), and Moore (personal communication).

The effect of performic acid oxidation shows that this treatment has a significant effect on the serine, threonine, tyrosine, phenylalanine, and histidine contents. Consistent with the destruction of these amino acids is the corresponding increase in the ammonia nitrogen figure. Previous authors (Toennies and Homiller 1942; Hirs 1954; Smith and Stockell 1954) have differed on what amino acids are destroyed, and it would appear to depend on the conditions of oxidation and on the amino acid sequence adjacent to the labile amino acid. Hirs (1954) noted that on oxidation of ribonuclease A at  $-10^{\circ}\text{C}$  with preformed performic acid, no effect on the amino acid composition was observed other than the conversion of cystine to cysteic acid and methionine to its sulphone. Smith and Stockell (1954), using different conditions of oxidation, reported partial destruction of phenylalanine and almost complete destruction of tyrosine. Blackburn and Lowther (1951) mentioned the possibility of destruction of threonine, in agreement with our results, which also confirm those of Toennies and Homiller (1942) and Sanger and Tuppy (1951) regarding the stability of

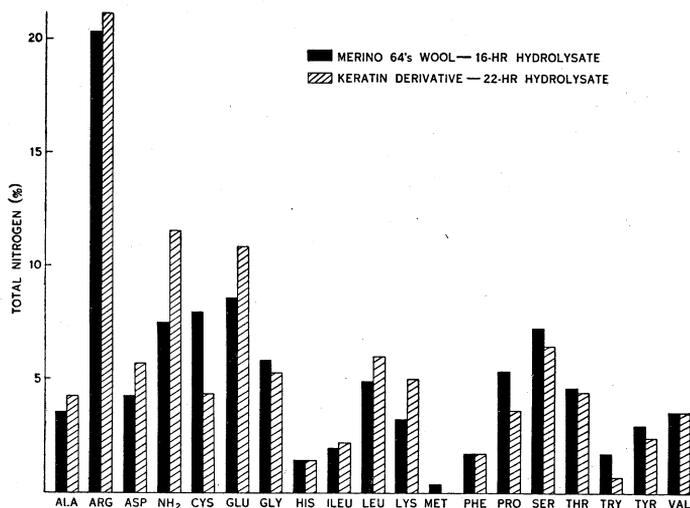


Fig. 2.—Comparison of the amino acid composition of a keratin derivative with that of the Merino 64's wool from which it was extracted. Figures represent percentages of total nitrogen.

the acidic and aliphatic neutral amino acids. Further work will be necessary to decide whether adequate correction factors can be applied to eliminate the need for a duplicate series of determinations on both oxidized and unoxidized samples, but from the present results it does not appear that this approach will be successful.

Differences in the amino acid composition of the 22-hr hydrolysate of the pure protein component and of a 16-hr hydrolysate of Merino 64's quality wool (taken from Table 3, Simmonds 1954a) are most readily seen by reference to the

block graph of Figure 2. The figure quoted in this for cystine (4.33 per cent.) is that obtained from the samples of wool protein which had been oxidized with performic acid. The difference of 6 hr in the hydrolysis times of the whole wool and the purified protein component would be expected from the results summarized in Table 1 and discussed above, to slightly affect the relative amounts of glutamic acid, serine, and threonine present in the two samples. This effect is small and it is considered that the graph clearly shows that the purified protein component appears to contain more of the acidic amino acids, aspartic and glutamic, and more leucine, lysine, and ammonia nitrogen, but less cystine, proline, serine, and tryptophan than the parent wool from which it was derived. This is in accord with recent theories on the structure of the cortex of Merino wool fibres (Horio and Kondo 1953; Mercer 1953; Fraser and Rogers 1953; Fraser, Lindley, and Rogers 1954), where it is assumed that the "S-" segment in the terminology of Fraser and Rogers (1953), containing fewer cystine disulphide cross linkages, is more susceptible to chemical attack.

In contrast to the nitrogen, the sulphur content has not been entirely accounted for. The sulphur figure (2.55 per cent.) exceeds that calculated from the cystine content (1.51 per cent.) by 1.04 per cent. The methionine contribution to this difference is likely to be small, and it is assumed that the remainder is derived from thioglycollic acid which is bound to some of the cysteine residues to form the mixed disulphide. No evidence was found during the analysis of the unoxidized protein samples, of peaks which could have been due to a mixed disulphide of cystine and thioglycollic acid, but it is possible that such a combination would have been ruptured during the hydrolysis with 6N HCl and reformed as cystine and the oxidized form of thioglycollic acid.

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