The Analysis of Hair Keratin

1. APPLICATION OF MICROBIOLOGICAL TECHNIQUES TO HYDROLYSATES OF HUMAN HAIR

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Analytical difficulties encountered during attempts to determine amino-acids in (or isolate them from) protein hydrolysates containing a large proportion of cystine first interested one of us (C.C.L.) nearly twenty years ago, but because the study was carried on sporadically, as a side-line, it progressed only slowly (Lucas & Beveridge, 1940; Beveridge & Lucas, 1944*a-c*). The development since about 1943 of microbiological assay procedures for amino-acids has made obsolete the laborious techniques previously used in studying protein hydrolysates. To get more complete and more reliable analytical data on the composition of human hair these newer methods have been applied to a freshly prepared hydrolysate of the same sample of cleaned hair as had been used by Beveridge & Lucas. The hair had meanwhile been stored in a tightly stoppered (plastic screw-cap) brown bottle in a dark cupboard. New analytical data for moisture (8.13 %), ash (0.51 %), and nitrogen, on moisture- and ash-free keratin (16.4 %) agreed well with the earlier determinations. Approximate values were obtained for most of the amino-acids in human hair keratin (Table 1); for comparison the values found some years ago by chemical procedures are included.

METHODS

Most of the amino-acids were determined by microbiological assay techniques, essentially as described by Henderson & Snell (1948); these were applied directly to the hydrolysate without removal of any cystine. Alanine was estimated by the method of Sauberlich & Baumann (1949) and serine was determined according to Stokes & Gunness (1945). Five organisms (shown in Table 1) were employed to assay the amino-acids. Standards were set up at five levels and tests at four levels, with tubes in triplicate in each case. Tryptophan was estimated chemically on unhydrolysed keratin by the method of Spies & Chambers (1949) which involves condensation with *p*-dimethylaminobenzaldehyde in 1*N*-H₂SO₄ and development of a blue colour upon treatment with NaNO₂. Cystine in the hydrolysate was estimated by Lugg's (1933) modification of Sullivan's (1926) procedure. The chemical methods used for the other amino-acids have been described in detail elsewhere (Beveridge & Lucas, 1944*a-c*), but the fourth column of Table 1 indicates the general nature of the procedures used in these determinations.

RESULTS AND DISCUSSION

Analytical values for nineteen of the commoner amino-acids in human hair, as determined in this laboratory (fourteen by chemical and sixteen by microbiological methods), are shown in Table 1. Those familiar with the fractionation of protein hydrolysates know that significant losses usually occur in the mother liquors during precipitation of copper and zinc salts and during recrystallization of the free amino-acids. The figures in column 5, Table 1, for alanine, the leucines and valine are therefore given in parentheses, with a plus sign, to indicate that while the chemical work established the presence of these amino-acids, the amounts isolated represent minimal values. It is unreasonable, therefore, to make comparisons between these figures and the doubtless more reliable data obtained by microbiological assay. The one serious discrepancy (on a percentage basis, if not in absolute amount) is in the case of histidine, where 1.2 % was found microbiologically and only 0.6 % by precipitation according to Kossel & Kutscher (1900-1) as silver salt, and re-precipitation as flavianate according to Vickery & Leavenworth (1927, 1928). The separation of histidine in the presence of much cystine appears to offer special difficulties. Argiris (1907-8) was unable to isolate any histidine from a similar type of hydrolysate (horse-hair). Vickery & Leavenworth (1929) found only 0.52 % of histidine in human hair. According to Dunn (1949) some laboratories fail to get satisfactory values in the microbiological estimation of arginine, histidine and lysine; no difficulties or sources of error were recognized in these determinations in our laboratory although the values for arginine and lysine were lower than by isolation. Significantly lower values (about 80 % of those estimated by isolation methods) were also obtained microbiologically for tyrosine. While the absolute magnitudes of the differences are small (at the worst less than 3 % of the keratin), these discrepancies are relatively large in percentage of the value found and would make a considerable difference in the molecular ratios if one were interested in calculating them. The fact that the values by microbiological

assay for the three amino-acids mentioned were smaller than those determined chemically might be explained either by inhibition of the assay organisms by the excessive amount of cystine in the hydrolysate or on the basis that some racemization had occurred during hydrolysis or, thirdly, that the fractions isolated by Beveridge & Lucas, in spite of good analytical values, were somewhat impure.

One is left with the impression that the higher values for arginine, lysine and tyrosine by the isolation procedures were probably due either to partial racemization giving low values in the microbiological assay, to extraneous amino-acids contaminating the fractions isolated, or to a combination of these effects. Unfortunately, suitable assay organisms are not known that respond equally well to both enantiomorphs of the amino-acids.

Table 1. *Analytical data obtained on human hair keratin*

(The values are reported on a moisture- and ash-free basis. (Letters in brackets indicate references.) A.T.C.C. = American Type Culture Collection, Washington D.C.)

Amino-acid	Test organism	%	Chemical method	%	Values reported by others
Alanine	1	2.8	Cu salt	(2.3 +)	6.9 (a)
Arginine	2	7.8	Flavinate	8.9	9.4 (b)
Aspartic acid	3	3.9	Ba, Cu salts	3.5	—
Cystine	—	—	Colour*	18.0	21.2 (c); 16.5 (d)
Glutamic acid	4	13.6	Ba salt, HCl	10.6	8.0 (a)
Glycine	3	4.1	—	—	9.1 (a); 4.3 (e)
Histidine	2	1.2	Flavinate	0.6	0.6 (e)
Hydroxyproline	—	—	Reineckate	0	—
Isoleucine	3	4.8 }	Crystallization	(7 +)	12.1 (a)
Leucine	4	6.4 }			
Lysine	3	1.9	Picrate	2.4	2.5 (d); 2.5 (e)
Methionine	2	0.7	Thiolactone†	0.8	—
Phenylalanine	3	2.4	—	—	2.6 (e)
Proline	3	4.3	Cu salt	4.3	—
Serine	5	10.6	HIO ₄ ‡	8.0	—
Threonine	2	8.5	—	—	—
Tryptophan	—	—	Colour§	1	1.6 (f); 0.7 (e); 1.1 (g)
Tyrosine	3	2.2	Crystallization	2.5	4.3 (g); 3.3 (a); 3.0 (e)
Valine	4	5.5	Cu, Zn salts	(1.1 +)	—
Melanin	—	—	—	1.6	—
Ammonia	—	—	—	1.42	—
N on inorg. ppts.	—	—	—	(1.13)	—

1, *Leuconostoc citrovorum* (A.T.C.C. 8081).

2, *Streptococcus faecalis* (A.T.C.C. 8043).

3, *Leuconostoc mesenteroides* P 60 (A.T.C.C. 8042).

4, *Lactobacillus arabinosus* 17-5 (A.T.C.C. 8014).

5, *Lb. delbrückii* LD5 (A.T.C.C. 9595).

* Lugg (1933).

† Kassel & Brand (1938).

‡ Nicolet & Shinn (1941).

§ Spies & Chambers (1949).

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(a) Buchtala (1913).

(b) Vickery (1940).

(c) Wilson & Lewis (1927).

(d) Vickery & Leavenworth (1929).

(e) Block, Bolling, Brand & Schein (1939).

(f) Sammartino (1922).

(g) Klinke (1925).

A mixture of amino-acids of the composition shown in Table 2, which, according to the findings, approximates a hair hydrolysate, was prepared and analysed microbiologically for arginine, glutamic acid, histidine and threonine. The recoveries were 99, 101, 101 and 97 %, respectively, of the amounts used. Cystine does not seem to exert any inhibitory influence under these conditions. Some other as yet unrecognized compound might, of course, have an inhibiting effect.

Most of the determinations were repeated several times on different hydrolysates, in some cases with two or even three different organisms. A few typical sets of results will indicate the variations encountered: glycine 3.95, 3.98, 4.09, 4.11; histidine 1.14, 1.16, 1.23, 1.26, 1.30; leucine 6.30, 6.37, 6.42; valine 5.17, 5.40, 5.41, 5.48, 5.64, 5.81. Glutamic acid values as estimated by *Lactobacillus arabinosus* and by *Leuconostoc mesenteroides* P 60 were essentially the same: 13.14, 13.19, 13.21, 13.48, 13.55,

13-70, 13-82, 13-90, 13-94 with the first organism and 13-23, 13-64 and 13-99 with the second. *Streptococcus faecalis* gave somewhat lower values, 12-12, 12-30 and 12-97.

Although microbiological methods for glycine, alanine, proline and serine have not proven entirely satisfactory (Dunn, 1949), in this instance the value for proline agrees with that determined chemically. The value found for serine by chemical means (periodate) is 25 % lower than that found using *Lactobacillus delbruckii* LD 5; it is difficult to decide which value may be nearer the truth.

The cystine value of 18.0 % was adopted after a study of its rate of liberation. Hair was boiled with 9N-hydrochloric acid (5 ml./g.) and free cystine was estimated at intervals in the hydrolysate. A maximal value of just over 18 % was obtained after 6 hr.: the value was 18.0 % at 10 hr. and 16.4 % after 24 hr. Merrill (1921) found maximal values for cystine in wool hydrolysates after boiling with acid for between 6 and 12 hr., and Rimington (1929) after 7 hr.: both noted that a slow decrease occurred on longer boiling. Sullivan & Hess (1937) found the cystine in casein to be completely liberated after 6-7 hr. boiling with 20 % (w/v) hydrochloric acid. Vickery & Leavenworth (1929) give a value of 16.5 % for the cystine content of human hair (determined after hydrolysis with 8N-hydrochloric acid for 8 hr.). If the higher figure of 18.0 % is adopted, and a value of 0.7 % for methionine is accepted, the sulphur content of the hair is practically accounted for (sulphur calculated from these data, 4.97; found, 5.00 %).

By microbiological procedures about 81 g. of material in the hydrolysate from 100 g. of hair keratin have been identified. By isolation or chemical methods approximately 71 g. were characterized; the absence of one amino-acid (hydroxyproline) appears to have been established by chemical means. Melanin and ammonia increased the material accounted for by another 3.0 g. The total weight of material identified is about 104 g. (The mean molecular weight of the constituent amino-acids is, by calculation, about 152 so that 100 g. of keratin on hydrolysis should yield about 112 g. of split-products.) The failure to identify about 8 g. of material out of the expected 112 g. has been a disappointment. The amounts of the amino-acids shown in Table 2 represent 85.0 % of the total nitrogen; ammonia and melanin account for another 7.7 %. Thus, it has been possible to account for about 93 % of the nitrogen of human hair keratin.

In the course of the chemical fractionation about 1.13 g. nitrogen, equal to 7 % of the total nitrogen, was found adsorbed to inorganic precipitates in a form not removable by washing with water or dilute acid. This nitrogen, of as yet unrecognized nature,

probably represents about 10 g. of amino-acids. Only if the bulk of the adsorbed nitrogen were in a form different from that of any of the 16 amino-acids determined microbiologically would one be justified in adding this 10 % of the material weight or 7 % of the total nitrogen to the 93 % of each of these constituents already identified.

Table 2. *Amino-acid content of human hair keratin*

	In hair keratin (%)	N (g./100 g.)	N (as % of total)
Alanine	2.8	0.44	2.68
Arginine	8.9	2.87	17.50
Aspartic acid	3.9	0.41	2.50
Cystine	18.0	2.10	12.81
Glutamic acid	13.6	1.29	7.87
Glycine	4.1	0.77	4.68
Histidine	1.2	0.33	2.01
Hydroxyproline	0	0.0	0
Isoleucine	4.8	0.51	3.11
Leucine	6.4	0.68	4.15
Lysine	1.9	0.36	2.22
Methionine	0.7	0.07	0.43
Phenylalanine	2.4	0.20	1.22
Proline	4.3	0.52	3.18
Serine	10.6	1.41	8.62
Threonine	8.5	1.00	6.10
Tryptophan	1	0.14	0.85
Tyrosine	2.2	0.17	1.04
Valine	5.5	0.66	4.02
Melanin	1.6	0.10	0.61
Ammonia	1.42	1.17	7.13
Unaccounted for	(8.2)	1.2	7.3
Total	112	16.4	100

Because different regions of the hair shaft may be distinguished histologically, it would appear reasonable to assume that different proteins may be concerned in its make-up. Consequently it would seem foolish to consider that the keratin material analysed represents a single compound. When the percentages found were divided by the respective molecular weights to get some idea of the relative frequency of occurrence of the amino-acids, a series of numbers not too far removed from simple ratios were obtained. Even if no assumption is involved as to the homogeneity of the material analysed, it is interesting to note that the frequency of occurrence of the amino-acids, on a molecular basis, appears to be roughly as follows: serine 32, glutamic acid 30, cystine 25, threonine 24, glycine 18, arginine 16, leucine 16, valine 16, isoleucine 12, proline 12, alanine 10, aspartic acid 10, phenylalanine 5,

lysine 4, tyrosine 4, histidine 2, methionine 2 and tryptophan 2.

SUMMARY

1. Values are given for nineteen of the common amino-acids in human hair keratin.

2. These account for about 90 % of the expected weight and 86 % of the nitrogen of the keratin;

melanin plus ammonia account for another 3 % of the weight and 7.7 % of the nitrogen.

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Further Isotopic Studies on Haemoglobin Formation in the Rat and Rabbit

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Recent work done *in vivo* with rabbits (Muir & Neuberger, 1950) and *in vitro* with nucleated red cells of birds (Radin, Rittenberg & Shemin, 1950) has shown that the α -carbon atoms of glycine molecules provide eight carbon atoms for the porphyrin moiety of haemoglobin. Earlier investigations (Wittenberg & Shemin, 1949; Muir & Neuberger, 1949) had produced evidence that all four nitrogen atoms of porphyrin are also specifically derived from glycine. The present paper is concerned in the first place with a comparison of the isotope contents of the porphyrin and glycyl residue in rabbit haemoglobin at different time

intervals after administration of methylene-labelled glycine. If glycine is indeed a specific precursor of porphyrin, it would be expected that the ratio of 'molar' radioactivities of porphyrin and globin glycine should approach or be equal to the value of eight, provided that the rate of blood formation is constant and that globin and porphyrin are made in the same cell or cell compartment, during the experiment, i.e. utilize the same glycine 'pool'. It is of course possible that the newly formed porphyrin or globin are diluted to different extents by unlabelled preformed material or that the average times required for the incorporation of the α -carbon atoms of glycine into the porphyrin and globin moieties of haemoglobin differ significantly. In

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