Proteins of Normal Hair and of Cystine-Deficient Hair from Mentally Retarded Siblings

BY R. J. POLLITT AND P. D. STONIER

Medical Research Council Unit for Metabolic Studies in Psychiatry, University Department of Psychiatry, Middlewood Hospital, P.O. Box 134, Sheffield S6 1TP, U.K.

(Received 20 November 1970)

1. *S*-Carboxymethylkerateines extracted from normal hair can be fractionated into high-sulphur and low-sulphur proteins similar to those obtained from sheep's wool. Normal human hair gives a major high-sulphur protein of higher molecular weight and *S*-carboxymethylcysteine content than any isolated from normal sheep's wool. 2. The proteins from cystine-deficient hair can also be divided into high-sulphur and low-sulphur proteins. There is a lower proportion of high-sulphur protein in cystine-deficient hair than in normal hair. 3. The high-sulphur proteins from cystine-deficient hair have an abnormal amino acid composition and in particular are lower in *S*-carboxymethylcysteine content than the corresponding proteins from normal hair. New components are present and the content of very high-sulphur proteins of high molecular weight is much decreased. The low-sulphur proteins of cystine-deficient hair are probably also deficient in *S*-carboxymethylcysteine. 4. The proteins of cystine-deficient hair probably resemble those in the normal hair root, except that disulphide-bridge formation has occurred.

A new syndrome has been described in a brother and sister with mental and physical retardation. They have brittle hair that shows a number of unusual morphological features and has an abnormal amino acid composition, with only half the normal cystine content (Pollitt, Jenner & Davies, 1968). This paper examines the proteins of this hair and of normal human hair in an attempt to explain this condition.

The proteins of sheep's wool are being studied intensively by several groups (Crewther, Fraser, Lennox & Lindley, 1965; Crewther, 1967). There has been relatively little work on human hair. Reduced and carboxymethylated proteins analogous to those obtained from wool have been prepared from hair and have been separated into high- and low-sulphur fractions (Crewther, Gillespie, Harrap & Inglis, 1966; Gillespie & Inglis, 1965). Electrophoretic patterns of various extracted human keratins have been obtained by several workers. The structure of hair seems to be broadly similar to that of wool, and we have used the methods developed for *S*-carboxymethylkerateines of wool in this study.

METHODS

Most of the detailed studies were done on hair from the female patient as hers was more readily available. The extraction and separation procedures are outlined in Scheme 1.

Hair was washed in several changes of light petroleum

(b.p. 40°-60°C), and then in three changes of 50% (v/v) ethanol and dried overnight. It was extracted with thioglycollate at pH 11 in the presence of 8 M-urea with a liquor/hair ratio of 100:1, and the mixture was treated with iodoacetate (Gillespie, 1964; Gillespie & Inglis, 1965). Insoluble material was removed by centrifugation and the proteins in the supernatant were recovered by dialysis and freeze-drying. The insoluble residue was washed well, dried and weighed.

Isoelectric precipitation was carried out by the methods of Gillespie (1964), sometimes in the presence of 0.5 M-KCl (Gillespie, O'Donnell & Thompson, 1962). Separations on Sephadex G-200 were in a buffer of 0.01 M-tris, 1 mM-EDTA, 0.5 M-KCl and 8 M-urea, adjusted to pH 7.4 with HCl (O'Donnell & Thompson, 1964, 1965). The column was 540 mm × 30 mm and fractions of 10 ml were collected. The extinction at 276 nm was determined for the contents of each tube, and the appropriate fractions were combined. Proteins were recovered by dialysis and freeze-drying. DEAE-cellulose chromatography was carried out on a column (290 mm × 13 mm) of preswollen microgranular DEAE-cellulose (Whatman DEAE-52) equilibrated with sodium acetate buffer, pH 4.5 and 1.02 (Gillespie, 1963). The protein was applied in pH 4.5 buffer and was followed by 29 ml of the same buffer. A linear gradient of 0.25 M-0.5 M-NaCl was then started with a flow rate of 12 ml/h. Fractions of 2.6 ml were collected and pooled and protein was isolated as before. The flow rate has a very marked effect on this separation and it is important that the column is not packed under pressure as usually recommended.

Polyacrylamide-gel electrophoresis was performed in an improvised vertical-plate apparatus (Akroyd, 1968) by using buffer systems based on those of Racusen &

Scheme 1. Extraction and separation procedures used to produce the major fractions used in this study. Fractions underlined have full amino acid analysis in the Tables; other fractions are in the text.

Calvanico (1964). The upper buffer was pH 8.3 and the gel and lower buffers were pH 8.9. The gels were 10 or 20% (w/v) acrylamide and sometimes contained 4M-urea. After electrophoresis, the gels were washed overnight in 10% (w/v) trichloroacetic acid and then stained in 0.1% Nigrosine.

Isoelectric focusing in polyacrylamide gel followed the method of Dale & Latner (1968) adapted for use with plates rather than tubes. Ampholytes in the range pH 3–6 (Ampholine; LKB-Producter AB, S-161 25 Bromma 1, Sweden) were used and the potential was applied for 29 h.

The proportions of the various fractions in the total hair extracts were calculated for several normal samples and for the patients' hair. The proportions of high-sulphur protein to low-sulphur protein were determined by weighing the freeze-dried IE-A1 and IE-B1 fractions. An alternative method based on the extinctions at 276 nm, with correction for the different tyrosine contents of the two fractions, gave similar values but with a slightly greater scatter. The high-sulphur protein fractions were subdivided in the ratios of the integrated extinctions of sections of the elution curves from the DEAE-cellulose columns.

Hydrolysis of proteins was carried out in evacuated tubes for 18 h at 106°C in 5.7M-HCl. A Locarte amino acid analyser was used, with a single-column programme. All peaks were adequately resolved. The results were not corrected for losses during hydrolysis.

The results in the tables are expressed as percentages of total nitrogen (excluding ammonia) recovered from the column. In some of the tables, results from several different samples have been expressed as an average and standard deviation. Although in most cases the number of samples is too small to give the standard deviation much statistical meaning, it serves to indicate the overall consistency of any particular fraction from different hair samples.

Free thiol groups were determined from the uptake of phenyl[²⁰³Hg]mercuric acetate by the method of Leach, Meschers & Springell (1966). Samples of hair (about 10 mg) were incubated with the phenylmercuric acetate solution for 46 h at 25°C and the uptakes calculated from the loss of radioactivity from the solution. The radioactivities of the solutions were counted as emulsions in Triton-toluene in a Packard Tri-Carb scintillation spectrometer.

Optical rotatory dispersion was determined on a Bendix Polaromatic 62 photoelectric polarimeter. α -Helix contents were calculated by using the method of Crewther *et al.* (1966). Sedimentation determinations were carried out in a Beckman model E analytical ultracentrifuge at 20°C.

RESULTS

Extraction. The amino acid analyses of samples of hair from ten normal subjects and the two patients are summarized in Table 1. The extraction procedure solubilized up to 80% of the hair, though this varied considerably from one subject to another. In both the patients and normal subjects the extract contained slightly more cystine (analysed as *S*-carboxymethylcysteine) than the residue, in agreement with the preferential solubilization of high-sulphur proteins observed by Gillespie (1962). The residues were also significantly richer in glycine and methionine (Table 1).

Isoelectric precipitation. The isoelectric precipitate (IE-A1) was low in *S*-carboxymethylcysteine whereas the supernatant (IE-B1) was rich in *S*-carboxymethylcysteine, serine, proline and threonine (Table 2).

The supernatant had a reasonably consistent composition for the different normal hair samples,

Table 1. *Amino acid composition of whole hair, hair extracts and residues*

	Normal hair Av. $\pm$ s.d. (10)	Patients' hair	Normal (av. of 3)		Patient	
			Extract	Residue	Extract	Residue
Asp	4.3 $\pm$ 0.2	6.3	4.4	4.5	6.5	5.8
Thr	6.0 $\pm$ 0.2	4.4	6.4	5.1	4.9	4.2
Ser	9.6 $\pm$ 0.3	7.7	8.8	9.7	7.9	8.7
Glu	9.7 $\pm$ 0.2	11.4	9.8	9.6	10.1	9.6
Pro	7.3 $\pm$ 0.3	5.8	6.4	6.4	4.5	6.1
Gly	5.1 $\pm$ 0.4	5.0	4.3	6.1	5.0	6.2
Ala	3.8 $\pm$ 0.1	5.1	3.4	4.7	5.0	5.8
SCM-Cys*	14.4 $\pm$ 0.4†	6.7†	15.6	13.8	7.8	6.8
Val	4.4 $\pm$ 0.1	4.7	4.3	5.1	5.0	5.8
Met	0.39 $\pm$ 0.08	0.70	0.24	0.53	0.34	0.94
Ile	2.2 $\pm$ 0.1	3.0	2.2	2.6	3.0	3.1
Leu	5.1 $\pm$ 0.1	7.2	5.0	5.4	7.3	6.4
Tyr	1.7 $\pm$ 0.1	2.1	1.8	1.8	2.1	2.2
Phe	1.4 $\pm$ 0.1	1.9	1.3	1.5	1.9	2.3
His	2.1 $\pm$ 0.2	3.0	2.0	2.0	2.7	4.0
Lys	4.3 $\pm$ 0.4	6.9	3.9	5.9	6.1	8.7
Arg	18.3 $\pm$ 0.6	18.1	20.1	15.5	20.0	13.5

* SCM-Cys: *S*-carboxymethylcysteine.

† Cystine.

Table 2. *Amino acid composition of isoelectric precipitation fractions*

	Precipitate IE-A1		Precipitate IE-A5		Supernatant IE-B1		Supernatant IE-B2	
	Normal Av. $\pm$ s.d. (3)	Patient	Normal Av. $\pm$ s.d. (3)	Patient	Normal Av. $\pm$ s.d. (4)	Patient	Normal Av. $\pm$ s.d. (4)	Patient
Asp	6.3 $\pm$ 0.5	7.3	7.2 $\pm$ 0.3	8.1	1.9 $\pm$ 0.2	3.2	2.2 $\pm$ 0.2	4.7
Thr	4.7 $\pm$ 0.7	4.0	4.0 $\pm$ 0.1	3.9	8.8 $\pm$ 0.3	8.2	9.2 $\pm$ 0.3	5.8
Ser	7.5 $\pm$ 0.4	6.8	7.3 $\pm$ 0.2	7.1	10.4 $\pm$ 0.2	11.4	10.7 $\pm$ 0.2	10.8
Glu	12.7 $\pm$ 0.7	13.7	14.3 $\pm$ 0.7	15.0	6.7 $\pm$ 0.1	6.9	6.3 $\pm$ 0.3	5.0
Pro	4.3 $\pm$ 0.8	3.4	2.8 $\pm$ 0.3	2.5	9.7 $\pm$ 0.3	9.3	9.2 $\pm$ 0.2	8.4
Gly	4.3 $\pm$ 0.2	4.4	3.7 $\pm$ 0.0	3.7	4.2 $\pm$ 0.2	7.2	4.9 $\pm$ 0.3	9.2
Ala	4.9 $\pm$ 0.4	5.8	5.5 $\pm$ 0.2	5.8	1.5 $\pm$ 0.1	2.7	1.8 $\pm$ 0.2	5.0
SCM-Cys*	8.6 $\pm$ 2.1	4.6	5.2 $\pm$ 0.2	2.9	25.0 $\pm$ 0.6	16.5	24.1 $\pm$ 0.4	8.4
Val	4.5 $\pm$ 0.1	4.9	4.6 $\pm$ 0.1	4.5	3.9 $\pm$ 0.1	5.4	3.7 $\pm$ 0.2	4.3
Met	0.35 $\pm$ 0.04	0.44	0.41 $\pm$ 0.01	0.52	0.07 $\pm$ 0.02	0.14	0.11 $\pm$ 0.02	0.21
Ile	2.9 $\pm$ 0.1	3.2	3.1 $\pm$ 0.1	3.2	1.3 $\pm$ 0.0	2.4	1.4 $\pm$ 0.0	2.9
Leu	7.6 $\pm$ 0.8	8.4	8.6 $\pm$ 0.1	9.0	2.1 $\pm$ 0.2	3.4	2.2 $\pm$ 0.1	3.9
Tyr	2.1 $\pm$ 0.1	2.3	2.2 $\pm$ 0.1	2.0	1.3 $\pm$ 0.1	1.6	1.5 $\pm$ 0.0	2.2
Phe	1.6 $\pm$ 0.0	1.9	1.7 $\pm$ 0.0	1.8	0.9 $\pm$ 0.0	1.7	1.0 $\pm$ 0.1	2.9
His	2.1 $\pm$ 0.2	1.8	2.2 $\pm$ 0.3	2.6	2.7 $\pm$ 0.2	3.0	2.2 $\pm$ 0.2	1.9
Lys	5.7 $\pm$ 0.6	6.6	6.5 $\pm$ 2.0	7.6	1.1 $\pm$ 0.1	1.7	1.1 $\pm$ 0.1	4.0
Arg	19.9 $\pm$ 1.6	20.3	20.8 $\pm$ 0.6	19.9	18.6 $\pm$ 0.7	15.3	18.6 $\pm$ 0.2	20.4

* SCM-Cys: *S*-carboxymethylcysteine.

but the precipitate IE-A1 was rather variable. A further precipitation step on IE-A1 of normal hair gave a supernatant (IE-B2) of similar analysis to IE-B1 (Table 2). The precipitate is thus fairly heavily contaminated with proteins that more properly belong to the supernatant fraction. The low-sulphur (IE-A) proteins from one sample of normal hair were precipitated for a total of eight times; the *S*-carboxymethylcysteine values fell from an initial 9.12 to 5.71, 5.01, 4.73, 4.42 and 3.61 after 3, 5, 6, 7 and 8 precipitations respectively. Other amino acids changed correspondingly. A sample from the patient similarly treated gave *S*-carboxymethylcysteine values of 4.63, 3.12, 2.87, 3.49, 3.39 and 3.42. In both cases the final precipitation was in the presence of 0.5 M-potassium chloride, which with wool proteins decreases the amount of high-sulphur protein precipitated with the low-sulphur protein (Gillespie *et al.* 1962). Analyses of the five-times-precipitated protein (IE-A5) from three normal subjects and the patient are shown in Table 2.

To investigate the effect of 0.5 M-potassium chloride in the buffer used for isoelectric precipitation, a sample of normal extract and extract from the patient's hair were each divided into two. One half of each extract was precipitated three times with 0.5 M-potassium chloride in the buffer and the other half without potassium chloride in the buffer. No significant differences were seen between the two parts of each extract, though the fractions from the patient's hair contained less *S*-carboxymethylcysteine than the normal hair as before.

Fig. 1. Chromatography on Sephadex G-200 of whole-hair extract from normal subject (----) and patient (—). For details see the text.

Sephadex chromatography. The normal whole-hair extract when chromatographed on Sephadex G-200 gave four distinct peaks (Fig. 1). Fraction S4 consisted of low-molecular-weight materials that could be removed by prolonged dialysis. Fractions S2 and S3 were purified by rechromatography. Their amino acid analyses and those of unpurified fraction S1 are shown in Table 3. Fraction S2 had a similar analysis to fraction IE-A1, which is known to be contaminated (in normal subjects) with high-sulphur proteins. Fraction S3 was less high-sulphur in character than the corresponding fraction IE-B.

The proteins in fraction S2 were fractionated by isoelectric precipitation to give fractions designated as S2-A and S2-B for the precipitate and super-

Table 3. *Amino acid composition of Sephadex G-200 fractions*

	Fraction S1		Fraction S2		Fraction S3	
	Normal Av. $\pm$ s.d. (3)	Patient	Normal Av. $\pm$ s.d. (3)	Patient	Normal Av. $\pm$ s.d. (3)	Patient
Asp	4.9 $\pm$ 0.1	6.9	6.3 $\pm$ 0.1	7.7	2.5 $\pm$ 0.0	4.1
Thr	5.6 $\pm$ 0.3	4.1	4.9 $\pm$ 0.1	4.1	8.3 $\pm$ 0.1	7.4
Ser	8.8 $\pm$ 0.6	7.3	7.7 $\pm$ 0.1	7.2	10.0 $\pm$ 0.3	9.8
Glu	10.9 $\pm$ 0.4	13.1	12.6 $\pm$ 0.1	14.4	7.2 $\pm$ 0.1	7.8
Pro	5.1 $\pm$ 0.4	3.5	4.7 $\pm$ 0.2	4.4	9.6 $\pm$ 0.5	8.3
Gly	4.2 $\pm$ 0.1	4.8	3.9 $\pm$ 0.1	4.0	4.8 $\pm$ 0.3	7.0
Ala	4.0 $\pm$ 0.2	5.9	4.7 $\pm$ 0.2	5.6	2.1 $\pm$ 0.1	3.5
SCM-Cys*	12.4 $\pm$ 0.8	4.6	8.8 $\pm$ 0.4	4.2	22.0 $\pm$ 0.3	11.6
Val	4.4 $\pm$ 0.1	4.7	4.4 $\pm$ 0.1	4.8	4.0 $\pm$ 0.1	5.3
Met	0.31 $\pm$ 0.03	0.43	0.28 $\pm$ 0.03	0.40	0.12 $\pm$ 0.03	0.25
Ile	2.5 $\pm$ 0.1	3.1	2.7 $\pm$ 0.0	3.2	1.6 $\pm$ 0.0	2.7
Leu	5.6 $\pm$ 0.2	7.9	7.3 $\pm$ 0.2	8.9	2.9 $\pm$ 0.1	4.6
Tyr	1.8 $\pm$ 0.0	1.9	1.9 $\pm$ 0.0	2.1	1.4 $\pm$ 0.1	1.8
Phe	1.4 $\pm$ 0.1	1.7	1.6 $\pm$ 0.1	1.7	1.2 $\pm$ 0.1	2.1
His	2.3 $\pm$ 0.4	2.1	2.1 $\pm$ 0.2	1.9	2.9 $\pm$ 0.2	4.3
Lys	4.8 $\pm$ 0.3	7.0	5.4 $\pm$ 0.0	7.1	1.7 $\pm$ 0.2	3.3
Arg	21.0 $\pm$ 0.4	21.0	20.9 $\pm$ 0.3	18.5	17.7 $\pm$ 0.4	16.3

* SCM-Cys: *S*-carboxymethylcysteine.Table 4. *Amino acid analyses of purified fractions*

	Fraction S1-A5		Fraction S2-A5		Supernatant S2-B
	Normal (1)	Patient	Normal (av. of 3)	Patient	Patient
Asp	7.4	7.5	7.7	8.5	4.9
Thr	3.5	3.6	4.0	4.0	5.6
Ser	6.7	6.2	6.6	7.2	10.0
Glu	14.4	14.0	14.8	14.8	5.3
Pro	4.4	3.9	2.4	2.4	8.1
Gly	3.8	4.1	3.8	3.3	10.8
Ala	5.8	6.9	5.7	6.1	5.2
SCM-Cys*	4.2	2.1	3.9	2.4	7.0
Val	4.8	5.2	4.7	4.9	4.6
Met	0.39	0.52	0.37	0.46	0.24
Ile	3.2	3.2	3.1	3.4	3.0
Leu	8.2	8.5	8.9	9.3	4.4
Tyr	1.4	1.8	2.0	1.8	2.1
Phe	1.6	2.0	1.5	1.6	2.5
His	2.0	2.2	2.0	3.0	1.8
Lys	6.6	7.2	6.8	6.8	4.2
Arg	21.7	21.0	21.4	20.1	20.3

* SCM-Cys: *S*-carboxymethylcysteine.

nantant respectively. The analysis of S2-B (three normal samples) was virtually identical with that of IE-B2; the precipitation step was repeated on fraction S2-A a further four times to give fraction S2-A5 (Table 4). Similarly S1 was reprecipitated to give fraction S1-A5 (Table 4). In both cases the reprecipitation steps decreased the *S*-carboxymethylcysteine contents very considerably.

In view of this apparent overlap of the high-sulphur fractions into the S1 and S2 regions of the Sephadex chromatogram a sample of normal IE-B was run down a Sephadex G-200 column. A considerable amount of material was present in the S1 and S2 regions, and that in the S2 region had a higher *S*-carboxymethylcysteine content than that in the S3 region.

Fig. 2. Separations on DEAE-cellulose at pH 4.5 of high-sulphur proteins (fraction IE-B1) from normal hair (-----) and patient's hair (——). ———, NaCl concentration in influent buffer. For details see the text.

Table 5. Amino acid analyses of major DEAE-cellulose fractions

	Fraction DEAE-C		Fraction DEAE-D		DEAE-D from fraction S2-B Normal
	Normal Av. $\pm$ s.d. (4)	Patient	Normal Av. $\pm$ s.d. (4)	Patient	
Asp	2.5 $\pm$ 0.1	3.6	1.4 $\pm$ 0.1	1.5	1.4
Thr	8.8 $\pm$ 0.2	8.8	9.2 $\pm$ 0.2	7.3	9.4
Ser	9.8 $\pm$ 0.2	10.5	11.9 $\pm$ 0.2	14.7	11.6
Glu	5.9 $\pm$ 0.2	6.4	7.6 $\pm$ 0.2	9.1	7.8
Pro	10.7 $\pm$ 0.4	9.4	9.1 $\pm$ 0.3	8.9	9.2
Gly	4.9 $\pm$ 0.1	7.5	4.0 $\pm$ 0.3	9.4	4.0
Ala	1.9 $\pm$ 0.1	2.8	1.2 $\pm$ 0.0	2.3	1.3
SCM-Cys*	21.2 $\pm$ 0.6	14.5	26.6 $\pm$ 0.7	18.3	27.3
Val	4.4 $\pm$ 0.2	6.0	3.4 $\pm$ 0.1	3.9	3.5
Met	0.15 $\pm$ 0.03	0.14	0.0	0.0	0.0
Ile	1.6 $\pm$ 0.2	2.5	1.1 $\pm$ 0.1	1.9	1.1
Leu	3.1 $\pm$ 0.2	4.0	1.3 $\pm$ 0.1	1.7	1.3
Tyr	1.1 $\pm$ 0.1	1.5	1.3 $\pm$ 0.1	1.4	1.3
Phe	0.9 $\pm$ 0.0	1.6	0.9 $\pm$ 0.1	2.1	1.0
His	2.8 $\pm$ 0.8	2.8	2.2 $\pm$ 0.2	3.6	2.3
Lys	1.2 $\pm$ 0.1	1.5	1.0 $\pm$ 0.1	2.6	1.0
Arg	19.0 $\pm$ 0.3	16.7	18.1 $\pm$ 0.4	11.5	18.9

* SCM-Cys: *S*-carboxymethylcysteine.

The extract from the patient's hair gave a similar pattern to that from normal subjects on Sephadex G-200 (Fig. 1) but with a much decreased proportion of material in the S3 fraction. Fractions S1, S2 and S3 all had lower *S*-carboxymethylcysteine contents than the corresponding fractions from normal hair (Table 2). This difference from normal subjects

persisted in fractions S1-A5 and S2-A5. Fraction S2-B from the patient's hair (Table 4) resembled fraction IE-B2 from the patient (Table 2). However, whereas in normal subjects both S2-B and IE-B2 are generally like IE-B1, in the patient there are very marked differences.

DEAE-cellulose. The proteins in fraction IE-B

Table 6. *Amino acid analyses of minor DEAE-cellulose fractions*

	Normal (av. of 2)					Patient				
	C-1	C-2	C-3	C-4	C-5	C-1	C-2	C-3	C-4	C-5
Asp	3.3	3.3	3.2	2.9	1.9	2.8	2.9	3.9	3.8	1.8
Thr	4.2	5.4	8.7	9.2	8.6	4.8	7.4	9.5	8.1	7.3
Ser	12.0	15.8	9.9	9.5	10.1	11.3	11.8	11.0	9.5	13.0
Glu	5.3	3.1	5.2	6.0	6.3	3.1	5.0	7.1	6.3	8.9
Pro	5.6	7.8	10.5	11.1	9.8	6.3	8.2	7.7	11.5	11.1
Gly	13.9	13.2	6.4	5.3	4.1	14.2	10.2	8.7	6.2	6.8
Ala	7.8	5.0	1.3	1.7	1.4	6.3	4.0	2.8	2.6	2.9
SCM-Cys*	4.2†	5.5	15.9	20.4	25.5	5.2‡	8.0	11.3	15.7	18.0
Val	3.2	4.8	4.8	4.4	4.0	3.4	6.9	6.8	5.0	4.8
Met	0.0	0.0	0.24	0.18	0.07	0.14	0.1	0.1	0.3	0.13
Ile	2.2	2.3	1.8	1.5	1.4	3.1	2.8	2.7	2.2	2.0
Leu	2.5	3.0	4.0	3.0	2.1	2.6	2.9	3.6	5.0	2.5
Tyr	0.0	1.8	1.3	1.2	1.2	2.1	2.2	1.6	1.4	1.6
Phe	2.4	2.2	1.2	0.9	0.7	3.2	2.0	1.6	1.5	1.7
His	12.4	5.5	3.7	2.7	2.6	4.6	2.1	2.5	4.2	3.0
Lys	2.4	0.9	1.9	1.3	1.0	2.1	1.2	1.1	2.6	2.0
Arg	18.7	20.6	20.1	18.9	19.3	24.7	24.2	18.1	14.1	12.5

* SCM-Cys: *S*-carboxymethylcysteine.

† Cysteic acid.

‡ 2.4 as cysteic acid

Fig. 3. Composition of hair extracts. The total extract was divided into high-sulphur and low-sulphur portions by isoelectric precipitation. The high-sulphur portion is subdivided in the ratios of the extinctions of the appropriate fractions from the DEAE-cellulose column (Fig. 2).

were separated further on a column of DEAE-cellulose to give two major fractions, DEAE-C and DEAE-D (Fig. 2 and Table 5). In two normal subjects and the patient, DEAE-C was subdivided into five fractions, which were analysed separately (Table 6). The quantitative separation of the hair

extracts into fractions IE-A and IE-B and the subdivision of fraction IE-B into the DEAE-fractions are summarized in Fig. 3.

The elution pattern from DEAE-cellulose of fraction S3 from normal subjects was similar to that of normal fraction IE-B except that the C5 and D peaks were largely missing. On DEAE-cellulose, normal fraction S2-B showed a very large DEAE-D peak (analysis Table 5) preceded by a sharp peak probably corresponding to fraction C5.

Polyacrylamide-gel electrophoresis. In a 10% gel in the absence of urea, the unaggregated low-sulphur proteins were resolved into two major bands. When 4M-urea was incorporated into the gel, a complex pattern of at least four closely spaced bands was seen. In both cases the abnormal proteins were indistinguishable from normal. Isoelectric focusing in a 5% gel with ampholine buffers in the range pH 3–6 gave only one band from fraction IE-A5, with identical isoelectric point for both normal and patient's proteins.

The high-sulphur proteins from the DEAE-cellulose column were examined by electrophoresis in 20% polyacrylamide gel without urea. Fractions C1 and C2 gave only faint indications of discrete bands. Fractions C3, C4, C5 and D were all heterogeneous, giving several bands, and there were important differences between the corresponding fractions from normal and the patient's hair (Fig. 4). A slow-moving band, present in fraction C3 from the patient

Fig. 4. Electrophoresis of DEAE-cellulose fractions on 20% polyacrylamide gel at pH 8.9. The origin is at the top and migration is towards the anode.

only, was particularly striking. Most of the proteins in fraction D were present as a diffuse intensely staining band near the origin. This band was largely missing in the patient's fraction D, though the faster moving bands were present. The slow-moving band was the high-molecular-weight high-sulphur protein found in fraction S2-B.

Physical measurements. Sedimentation coefficients were determined for fractions IE-A5 from normal and patient's hair. In the buffer used for Sephadex chromatography (with urea) protein from the normal subject gave 0.73S and the patient's protein gave 0.55S. In 0.05M-sodium tetraborate, pH 9.1, fraction IE-A3 from the normal, subject and the patient gave 2.91 and 2.03S respectively. The optical rotatory dispersion of samples of fraction IE-A5 from two normal subjects gave α -helix contents of 46.1 and 46.8%. A sample from the patient gave a helix content of 50.8%.

Free thiol groups. It was found that the uptake of the phenylmercuric acetate by the hair was somewhat slower than by wool. Consequently the incubation period was extended to 46h. Normal hair gave uptake of $21.8 \pm 4.0 \mu\text{mol/g}$ and the hair from the two patients gave 19.7 and 17.4 $\mu\text{mol/g}$.

DISCUSSION

Normal hair. There are considerable differences between the various amino acid analyses of human hair reported in the literature, possibly in part because of the different genetic and nutritional backgrounds of the subjects from whom the hair has been taken. Nevertheless, the analyses of hair from ten normal subjects in this study are fairly consistent and agree with those of Simmonds (1958).

The separation of the *S*-carboxymethylated hair proteins into fractions relatively rich in *S*-carboxymethylcysteine and poor in *S*-carboxymethylcysteine is analogous to that demonstrated for many other α -keratins. The amino acid analyses for our IE-A3 fractions from normal subjects correspond to those obtained by Crewther *et al.* (1966). The analyses of IE-B correspond moderately well to that of a human hair high-sulphur protein analysed by Gillespie & Inglis (1965); our samples had a slightly higher *S*-carboxymethylcysteine content and a higher content of phenylalanine and tyrosine.

An unsatisfactory feature of the preparation of the low-sulphur proteins is the continued lowering of *S*-carboxymethylcysteine content on successive precipitations. This could be due to loss of closely adherent high-sulphur proteins, but it may be that also small fragments high in *S*-carboxymethylcysteine are being lost by peptide-bond cleavage at each stage as was found by Corfield (1962) for keratoses obtained by oxidation of wool.

The elution pattern of whole-hair extract on Sephadex G-200 is similar to that shown for the *S*-carboxymethylkeratines of wool (O'Donnell & Thompson, 1964). In wool, peak S1 consists largely of aggregates of one of the components of the low-sulphur proteins. Unaggregated low-sulphur proteins are present in S2 and high-sulphur proteins in S3. However, normal human hair also has high-sulphur protein (DEAE-D) in the S2 region, which accounts for the high *S*-carboxymethylcysteine content of fraction S2 before isoelectric precipitation. This high-sulphur protein has a higher *S*-carboxymethylcysteine content than any protein from normal sheep's wool, but corresponds to the components formed during the sulphur enrichment of wool by the infusion of sulphur-containing amino acids into the abomasum of the sheep (Gillespie & Reis, 1966). Unlike our fraction DEAE-D, this protein contains no phenylalanine and has a very low aspartic acid content. The molecular weights of high-sulphur proteins from wool increase with increasing *S*-carboxymethylcysteine content. The dietary-enrichment protein (*S*-carboxymethylcysteine content 27.2) has a molecular weight of 37000 compared with a molecular weight of 23000 for SCMK-B2, a highly purified wool-protein fraction with an *S*-carboxymethylcysteine content of 23.6 (Lindley, Gillespie & Haylett, 1967). The occurrence of our DEAE-D protein (*S*-carboxymethylcysteine content 27.3) in the S2 region of the Sephadex chromatogram and the high-sulphur proteins of overall *S*-carboxymethylcysteine content of 22.0 in the S3 region indicates that there is a general relationship between *S*-carboxymethylcysteine content and molecular weight in the high-sulphur proteins of human hair also.

The significant difference in the proline contents

of S1-A5 and S2-A5 suggest that, as in wool, the aggregates are formed predominantly from only one of the low-sulphur proteins, though our experiments have not ruled out the possibility that S1 of human hair may be an entirely different high-molecular-weight protein.

Abnormal hair. The hair from the two patients is strikingly abnormal in amino acid composition. Only three of the amino acids are within 2 standard deviations of the normal values. The cystine content is only half that of normal hair, 22 standard deviations from the normal mean. As in normals there is a slight discrepancy between the amino acid analyses of the extract and the residue, but it appears that a representative selection of proteins has been solubilized. Both fractions IE-A and IE-B are lower in *S*-carboxymethylcysteine than the corresponding fractions in normal subjects, so that the overall hair analysis is not due simply to a lack of normal high-sulphur protein as was originally postulated (Pollitt *et al.* 1968); the proportion of high-sulphur protein (IE-B) is decreased additionally, however.

In view of the unsatisfactory nature of the isolation procedure for low-sulphur proteins, the significance of differences in *S*-carboxymethylcysteine content between the patients and normal subjects in the earlier precipitation stages might be questioned. However, this difference persists after eight precipitations and is seen also in fractions S1-A5 and S2-A5. In the later stages of these 'purifications' some of the samples from the patient began to show signs of degradation, judged by the appearance of lower-molecular-weight material on polyacrylamide-gel electrophoresis. This may also account for the drop in *S*-carboxymethylcysteine values, followed by a rise on further reprecipitations seen in the IE-A fractions from the patient. In the patient's IE-A5 only *S*-carboxymethylcysteine, aspartic acid, methionine, leucine, tyrosine and phenylalanine are different from the normal by 2 standard deviations. In the case of the last four at least, some of this apparent significance may be spurious owing to the small number of normal samples, though similar differences were seen in the two preparations of fraction IE-A3 from normal and patient's hair carried out to investigate the effects of potassium chloride on the separation.

The lower sedimentation coefficient of the low-sulphur proteins from the patient's hair is consistent with the loss of a small portion of the normal molecule. To account for the analysis, this portion would have to be rich in *S*-carboxymethylcysteine and poor in aspartic acid, compared with low-sulphur proteins; that is a high-sulphur type of fragment. Such fragments have been isolated from one of the low-sulphur proteins of wool (Crewther & Harrap, 1967; O'Donnell, 1969). They have largely

the random-coil configuration and the low-sulphur protein of the patient's hair (IE-A5) has a higher helix content than that of a normal hair. Similarly, the α -helix content of low-sulphur *S*-carboxymethylkeratines from a variety of species increases with decreasing *S*-carboxymethylcysteine content (Crewther *et al.* 1966). In view of these results, it is a little surprising that it is not possible to distinguish between the patient's and normal low-sulphur proteins on polyacrylamide-gel electrophoresis. It must be that the conformational and molecular-weight differences on the one hand compensate for the differences in charge on the other.

In normal subjects, fraction IE-B2 is very similar in analysis to fraction IE-B1 and apparently consists of high-sulphur protein that has co-precipitated with the low-sulphur protein at the first stage. In the patient, however, fraction IE-B2, obtained in about 10% yield, is quite unlike fraction IE-B1 in composition; it has an intermediate *S*-carboxymethylcysteine content and although the serine and proline contents are high, it also has high aspartic acid and alanine contents and low threonine content. This composition is unlike either the high-sulphur or the low-sulphur type of protein, and the glycine and phenylalanine contents are higher and the glutamic acid content is lower, than in either group of proteins. A very similar fraction (S2-B) is obtained by precipitating the S2 fraction from the patient's hair. In agreement with this, fraction IE-B2 from the patient's hair shows a slow-moving intensely staining band on electrophoresis on 20% polyacrylamide gel with very little contamination by the high-sulphur proteins. There are certain resemblances between this fraction and the non-helical tails isolated from wool low-sulphur protein by pronase digestion or by cyanogen bromide cleavage (O'Donnell, 1969), as these too have a high serine/threonine ratio and are rich in glycine and phenylalanine. The patient's IE-B2 protein does not, however, correspond in analysis to the missing fragment of the low-sulphur protein and its molecular weight seems too high. Its origin and the reason for its appearance largely in the second supernatant is obscure.

The high-sulphur proteins are more easily characterized than the low-sulphur proteins and there are undoubtedly large differences between the hair proteins of the patient and normal subjects. The abnormal hair gave virtually no high-sulphur high-molecular-weight protein corresponding to the major component of DEAE-D in normal subjects, and the electrophoretic patterns of fractions C3, C4 and C5 were quite different in the patient. The amino acid analyses of the DEAE fractions from the patient's hair were all lower in *S*-carboxymethylcysteine than the corresponding fractions from normal subjects. In both cases fraction C1 seems

to consist of oxidized and possibly degraded fragments.

Hair protein structure and synthesis. The defect in our patients' hair extends to most, if not all, of the extracted high-sulphur proteins, and probably to the low-sulphur proteins as well. We are not aware of any other inherited condition that affects the structure of so many proteins simultaneously. The unprecedented extent of these changes is probably due to the unusual nature of the hair keratin and its synthesis.

Underlying the methods used in this study is the assumption that the proteins extracted from the hair by reduction at high pH bear a close resemblance to actual proteins in hair itself, and that little degradation occurs in the extraction process. This view, as applied to wool, has been disputed by workers who suggest that the high-sulphur and low-sulphur fractions arise as artifacts by the hydrolysis of a larger molecule containing both high-sulphur and low-sulphur regions (Corfield, 1963; Corfield, Fletcher & Robson, 1967). Degradation has also been suggested as the reason for the large number of proteins found in reductive extracts of wool, and for the 'microheterogeneity' of some of the isolated low-sulphur fractions (Thompson & O'Donnell, 1967).

The sulphur content of the developing wool increases as it ascends the follicle. It is easier to envisage the abnormalities in our patient's hair as arising from some fairly simple defect that is in some way interfering with this sulphur-enrichment process. The proteins of the mature hair of our patient should resemble in some respects that of the normal hair root. The 'sulphur-enrichment' process in wool has been ascribed to a definite two-stage synthesis, low-sulphur proteins being synthesized first, and the high-sulphur proteins being added higher up the follicle. This theory is obviously not applicable to the single-protein theory of keratin structure, where it is suggested that additional high-sulphur sections are in some way added to the low-sulphur portion of the molecule at a later stage. A further theory (Fraser, 1969*a,b*) is that both high-sulphur and low-sulphur proteins are synthesized at the lower levels of the wool root, the sulphur increase being due to a difference in the proportion synthesized at higher levels.

Studies of the proteins actually present in wool roots have produced discordant results. Frater (1966) extracted low-sulphur proteins from the wool root that were very similar to the corresponding proteins of mature wool but had significantly lower *S*-carboxymethylcysteine contents and gave slightly different peptide 'maps' on tryptic digestion. The high-sulphur proteins showed greater differences between wool and wool roots. Downes, Ferguson, Gillespie & Harrap (1966) also showed that fractions

of wool-root high-sulphur protein from DEAE-cellulose columns had lower *S*-carboxymethylcysteine contents than corresponding fractions from wool high-sulphur proteins. In contrast, Fraser (1969*a,b*) obtained no overall differences in *S*-carboxymethylcysteine content between wool and wool-root high-sulphur proteins, though some of the individual fractions of the wool-root proteins did have low values. He also found that the high-sulphur proteins of the root appeared similar to those of mature wool on starch-gel electrophoresis except that the root proteins showed a slow-moving intensely staining band in the fraction eluted early from DEAE-cellulose. Fraction DEAE-C3 from the patients' hair shows a similar prominent band on electrophoresis (Fig. 4).

The proteins of our patients' hair show certain resemblances to those of wool roots. The observed changes do not, however, fit in completely with any of the current theories of wool synthesis. A condition where the structure of both low-sulphur and high-sulphur proteins (or protein portions) are affected simultaneously is to be expected only from the single protein theory but the nature of the changes, and the fact that fairly high-sulphur proteins are still synthesized, are not easily explained. At this stage it is probably not possible to interpret the defect in our patients' hair in terms of any of the suggested mechanisms of wool synthesis.

The formation of disulphide bridges in normal hair occurs in the upper region of the keratogenous zone. The absence of any excess of thiol groups in our patients' hair indicates that this process is occurring in the absence of the normal sulphur-enrichment process. In agreement with this, whereas much of the protein in the lowest levels of the normal hair root can be extracted with 8*M*-urea very little protein can be extracted from our patients' hair.

Electron-microscopic studies lend some support to the idea that the patients' hair may in some ways be immature as the cuticular cells show features characteristic of cuticular cells in the keratinizing zone of the root of normal hair (Robson & Sikorski, 1968). The cortex of the abnormal hair shows a typical microfibrillar texture. The microfibrils have often been considered to be low-sulphur protein embedded in high-sulphur matrix. The proportion of microfibril to matrix in the patient's hair is normal, which does not support a clear-cut morphological distinction of this sort.

Cause of the disease. In sheep the synthesis of high-sulphur proteins is to some extent controlled by the availability of sulphur-containing amino acids; supplementary feeding *per abomasum* leads to increased synthesis of a wool protein particularly high in cystine (Gillespie, Broad & Reis, 1969; Broad, Gillespie & Reis, 1970). A decreased

proportion of high-sulphur protein is found in steely wool associated with copper deficiency (Gillespie, 1964). In humans, kwashiorkor results in a decrease in the proportion of high-sulphur protein in the hair. The changes seem to affect many of the high-sulphur proteins (Gillespie, 1967), but are much less marked than that in our patients. These analogies suggest the possibility that the premature cessation of protein synthesis in our patients' hair may be due to some defect leading to a shortage of cystine in the keratinization zone. There is, however, no evidence to suggest any generalized defect in cystine transport or utilization in the amino acid pattern of the body fluids (Pollitt *et al.* 1968). The defect is probably one more specifically affecting the synthesis of hair keratin.

The distribution of hair-type keratin in the other tissues of the body is unknown. The fingernails of the patients are somewhat hypoplastic. They have a cystine content of 5.8 compared with 9.5 in a normal, and the other amino acids are present in abnormal amounts in a similar way to the hair. Both patients have signs of skin abnormality and the elder patient has started to have attacks of psoriasis. Whether epidermal keratin is homologous with hair keratin is uncertain, but it is tempting to ascribe these symptoms to a common defect. If hair-type keratin was widely distributed in normal human tissues, then its malformation in these patients might account for the general stunting of growth. A neurokeratin has been described, and defective formation of this could be invoked to explain the mental retardation. However, for such explanations to be convincing, much clearer criteria for chemical homologies between the various keratins are necessary.

A 4-year-old girl has recently been described whose hair has a low cystine content, and an overall amino acid analysis very similar to that of our patients' hair (Brown, Belser, Crounse & Wehr, 1970). This girl was not mentally or physically retarded. The morphological details of this hair apparently differ slightly from those of our patient. The hair shows a regularly alternating birefringence. Abnormal birefringence is also seen in our patients' hair, and appears to be related to the irregular fibre orientation. If the hair defects do prove identical, one must assume that the mental retardation is a variable feature of the disease, or purely fortuitous.

The isolated occurrence of this condition in two siblings suggested that it might be a recessive Mendelian character. Neither of the patients' parents, nor their brother, show any hair abnormality. The amino acid analyses for the whole hair are within the normal range. (For the mother, pubic hair was analysed as the head hair had been permanent waved and the cystine content was only 12.56.) Similarly, the quantitative division of the

extracted proteins into fractions IE-A, IE-B, S1, S2, S3 and the DEAE-cellulose fractions was normal. Analyses of fractions of the father's hair obtained by repeated isoelectric precipitation, by Sephadex chromatography and by DEAE-cellulose chromatography were also within the normal range. If this condition is a recessive trait, it is not expressed in the hair of the heterozygotes.

We thank Dr Margaret Davies and Professor F. A. Jenner for helping us in the study of this family, Dr D. N. Jones for the optical rotatory dispersion measurements, Professor A. Robson for helpful discussions and Mr L. Grant for preparing the figures. P. D. S. held a Medical Research Council Scholarship for Training in Research Methods.

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