

Sibs with Mental and Physical Retardation and Trichorrhexis Nodosa with Abnormal Amino Acid Composition of the Hair

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Trichorrhexis nodosa is a condition in which individual hairs develop areas where the shaft has split longitudinally into numerous small fibres. The hairs eventually break at these points leaving brush-like ends. Usually trichorrhexis nodosa results from mechanical trauma or harsh chemical treatment (Chernosky and Owens, 1966), but two inherited disorders, argininosuccinic aciduria (Allan *et al.*, 1958; Levin, Mackay, and Oberholzer, 1961; Westall, 1960) and a type of sex-linked neurodegeneration (Menkes *et al.*, 1962), are often associated with a very marked degree of trichorrhexis nodosa. This is a report of a brother and sister with another syndrome, showing mental and physical retardation, trichorrhexis nodosa, and other hair defects.

Case Reports

The two children are the second and third of a family of three. The parents were unrelated, and appear normal. The father, a farm worker, was born in 1936, and the mother in 1938. The mother's brother had Down's syndrome, but he died suddenly in 1965; no chromosome studies had been performed. The first child is a normal intelligent boy (N.C.) born in 1959.

Case 1. The second child (L.C.) was born in May 1961; her birthweight was 3.0 kg., body length 48 cm., and head circumference 33 cm. The mother had toxæmia of pregnancy, a severe antepartum haemorrhage, and the labour and delivery were long and difficult; in addition the child is reported to have been severely jaundiced for 10 days after her birth. She was not noted to be abnormal until 1 year old, when she showed no signs of wanting to stand or walk. She was obviously retarded, and in addition her hair was sparse, stubbly, and brittle. Her nails were ill-shaped and spoon-like.

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Her tongue was reported to have white plaques. She was at first thought to be suffering from the effects of cerebral anoxia at birth, which had caused brain damage, and the mother was therefore reassured about further pregnancies.

The patient was admitted for special studies in May 1966 aged 5 years. She was now a simple, fairly good-natured child, with an obviously vacant expression. She looked perhaps three years younger than her age. She was affectionate, was walking, and was feeding herself, but remained incontinent. She weighed 15.5 kg., her head circumference was 47 cm., and her body length was 91 cm.

No formal psychological testing was possible because of the short attention span. Her motor development was within the range of 18 months to 2 years. She had a wide-stanced gait, but could climb on to a chair. She could hold two cubes in one hand and return them to a container but not build with them. She could extend her arm to help when being dressed.

Her speech and understanding were at a level below that expected of a child aged 1 year. There was no recognizable speech.

She had short sparse eyebrows, and the scalp hair was 1-2 cm. long, except for areas of alopecia caused by rubbing (Fig. 1a). Apparently the hair condition had been progressively deteriorating, though with short remissions, since she was about 1 year old. The hair had a matted appearance and there was a follicular hyperkeratosis of the scalp and forehead.

The hair itself could easily be broken and showed classical trichorrhexis nodosa (Fig. 2a). In addition the shaft was often flattened in cross-section and sometimes twisted slightly in the long axis (pili torti). The hair surface was irregular, and scanning electron micrographs showed that the normal pattern of scales was almost completely absent (Fig. 3). The high-angle x-ray diffraction pattern was normal, but electron-microscopical examination of stained sections has shown several abnormalities (Sikorski and Robson, 1968).

She had cold blue extremities indicating a defective peripheral circulation, but examination, including ECG,

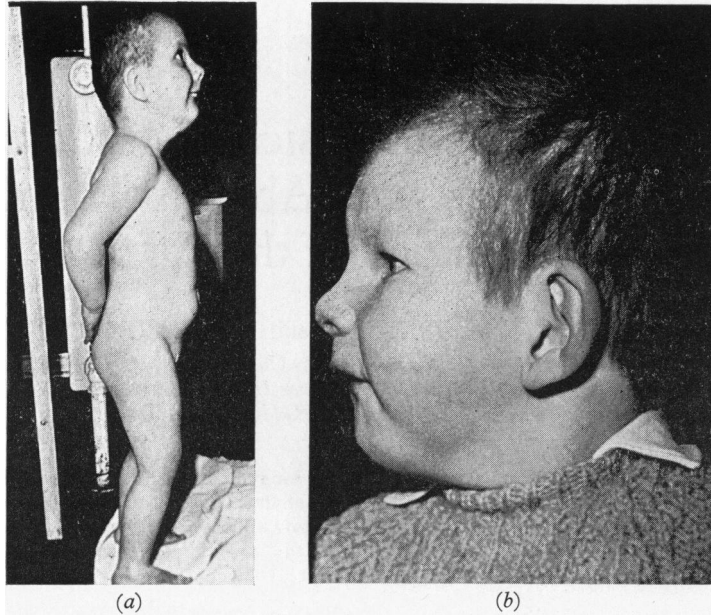


FIG. 1.—(a) Case 1, aged 5½ years and (b) Case 2, aged 3 years.

failed to reveal any other cardiovascular defects. The child was not hypotonic and examination of the central nervous system was normal.

Radiological studies. Skull was short based, with small vault (1175 ml. cranial capacity) approximately at

the 5th centile (Gordon, 1966). The sella turcica was bridged and small in size, the lateral area 40 sq.cm., and the sella index 1.4. The long bones and the bones of the hands and feet showed ossification compatible with a bone age of 1–1½ years; there were a number of disturbed growth lines in the metaphyses.

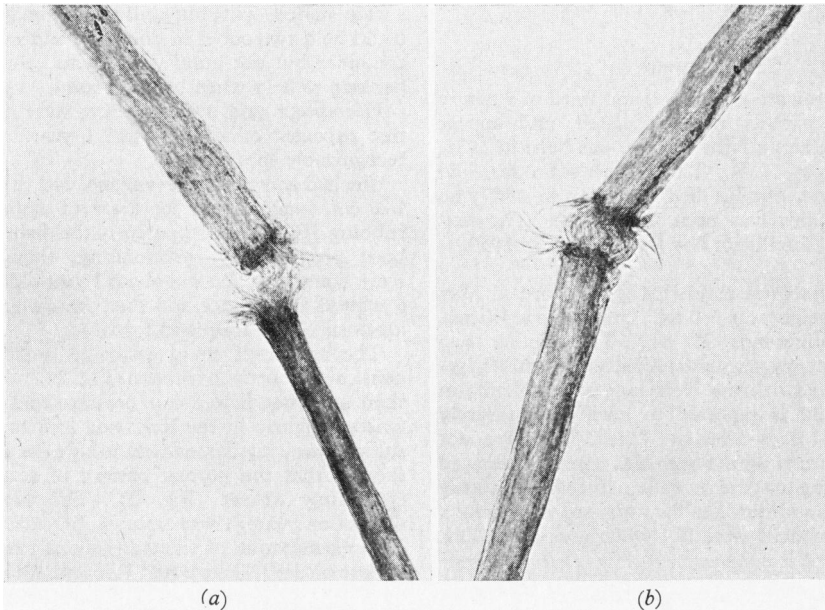


FIG. 2.—Light microscope photographs of the untreated hair. (a) Case 1; (b) Case 2.

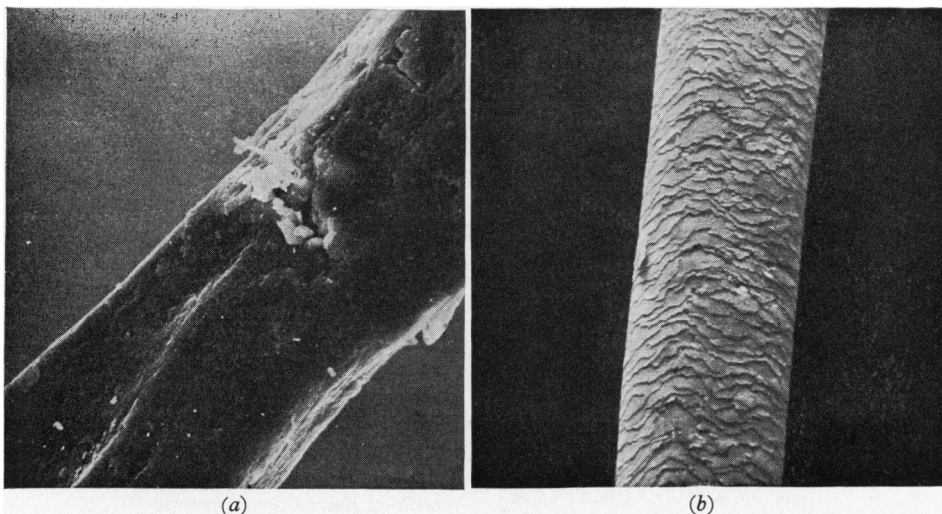


FIG. 3.—Stereoscan electromicrographs of hair (a) from Case 1 and (b) from a control subject.

Dental studies. The enamel of the teeth, though intact, was thin. Aged 5 the deciduous teeth had completely erupted and permanent dentition was well advanced. She displayed marked bruxism.

Chromosome studies. Buccal smears were of normal female type, and seven cells from a lymphocyte culture all contained 46 chromosomes and a normal female karyotype. Dermatoglyphs were normal.

Electroencephalogram. Non-specific abnormalities, with a paucity of faster rhythmic activity and an excess of slow components; there was no asymmetry.

Laboratory investigations. Urinalysis, serum proteins, and electrophoresis, serum cholesterol, electrolytes, uric acid, bilirubin, alkaline phosphatase, zinc sulphate precipitation test, thymol turbidity, and flocculation tests, SGPT, calcium, fibrinogen, blood urea, blood caeruloplasmin, ESR, Hb, red cell count, white cell count, platelet count, steroids, and pH were normal.

CSF showed no abnormalities of cell content, glucose, protein, chloride, or colloidal gold precipitation tests.

The serum inorganic phosphate was 4.8 and 5.25 mg./100 ml., outside the laboratory's range of 2–4 mg./100 ml.

Urinary phenolic acids after a plant-free diet were normal. The urinary amino acids were examined by high-voltage electrophoresis at pH 2.0, followed by chromatography in the second dimension using butanol-acetic acid-water (12:3:5). Argininosuccinic acid (Koch-Light Laboratories Ltd.) was also run as a standard. The pattern was normal; in particular, the cystine spot was of usual size and there was no trace of argininosuccinic acid. Plasma amino acids were examined by 2-dimensional thin-layer chromatography using systems I and II of von Arx and Neher (1963), after deproteinizing by picric acid or by gel filtration on Sephadex G25 and desalting on Dowex 50×8 resin.

The amino acids were present in normal amounts; in particular, the level of glutamic acid was not raised, and cystine was present.

CSF was deproteinized with picric acid, desalted, and subjected to thin-layer chromatography as before. The following amino acids presented clearly defined spots in descending order of intensity—glutamine, alanine, glycine, serine, leucines, glutamic acid, aspartic acid, lysine, phenylalanine, valine, histidine, threonine, arginine, cystine, and asparagine. Proline was also present but not easily compared with the other amino acids.

Faecal amino acids, examined by high-voltage electrophoresis, showed a normal pattern.

Whole blood -SH group levels determined by Ellman's (1959) method were within the normal range.

Chemistry of hair. A specimen of hair was hydrolysed in a sealed tube with 6N-hydrochloric acid and the resultant amino acids examined by 2-dimensional electrophoresis and chromatography. There was no trace of argininosuccinic acid or of its cyclic anhydrides (Westall, 1960; Ratner and Kunkemueller, 1966). It was noticed, however, that the proportions of the amino acids were different from those from a sample of normal hair so treated. The amino acid compositions of the hydrolysates were determined quantitatively on an amino acid analyser (Table I).

The low cystine content was confirmed using the Shinohara (1936) method, and specific chromatographic searches by Dr. D. J. Raven failed to reveal possible degradation products such as cysteic acid and lanthionine.

Case 2. A.C. was born in January 1964, weighed 3.130 kg., his body length was 48 cm.; he was cyanosed at birth and had a considerable ventricular septal defect. At 5 weeks, he had a folliculitis of his face and scalp:

TABLE I

Amino Acid Analyses of Hair and Hair Proteins: Results expressed as g. nitrogen per 100 g. nitrogen recovered from column (ammonia excluded)

	Case 1	Case 2	Control	Low-sulphur Protein*	High-sulphur Protein †
Asp ..	5.41	6.29	4.03	7.34	2.11
Thr ..	4.26	4.44	5.62	4.31	8.69
Ser ..	7.33	8.14	9.06	7.10	10.00
Glu ..	11.02	11.66	9.75	13.11	7.05
Pro ..	4.41	4.46	6.26	2.99	10.67
Gly ..	5.68	5.58	5.22	4.08	5.11
Ala ..	4.32	5.23	3.27	5.49	1.96
Val ..	3.67	4.34	3.43	4.83	4.38
‡-Cys ..	7.67‡	6.37	14.23	6.01	22.90
Met ..	0.77	0.77	0.49	0.34	—
Ile ..	2.08	2.51	1.66	2.89	1.55
Leu ..	7.36	7.37	5.49	8.06	2.45
Tyr ..	2.92	2.31	1.87	1.97	0.58
Phe ..	2.47	1.97	1.25	1.54	0.92
Lys ..	6.68	6.69	4.55	5.53	1.06
His ..	3.12	2.51	2.63	1.75	2.34
Arg ..	20.89	19.42	21.21	22.66	18.22

* Human hair, data from Crewther *et al.* (1966) recalculated.

† Human hair, data from Gillespie and Inglis (1965) recalculated.

‡ The Shinohara method on another sample of hair gave 9.0% cystine by weight, in agreement with these results.

head circumference was then 32 cm. It was immediately suspected that he was subnormal, and this diagnosis became increasingly apparent during the first year of life. At 9 months he was clearly retarded in growth but not in cardiac failure: head circumference was 41 cm. At 3 years (Fig. 1b) he can walk around holding the furniture and is saying the odd word, but inappropriately. He is less retarded than his sister.

His hair appears to be similar to his sister's and shows the same microscopical features (Fig. 2b). As yet, however, it is less severely affected. His skin is dry. His tongue has had frequent white plaques, he has koilonychia, and has had frequent attacks of flexural eczema. He has always had feeding problems and in particular vomits milk. His teeth are of particularly poor quality, with thin enamel like his sister's, but also with multiple cavities requiring repeated dental attention. His urinary amino acids were normal on examina-

TABLE II
The Family's Blood Groups

	Blood Groups			
Father ..	A	Rh + ve	CDe/cde	Ss
Mother ..	AB	Rh + ve	CDe/cde	S?
Case 1 ..	A	Rh + ve	CDe/cde	Ss
Normal sib ..	AB	Rh + ve	CDe/cde	S?
Case 2 ..	A	Rh - ve	cde/cde	Ss

Note: s could not be studied in the presence of AB. All the members were also MN, Kell-ve, Fy^a (Duffy)-ve.

tion. An amino acid analysis of his hair is given in Table I.

Table II gives the family blood groupings and confirms the reported relationships.

Discussion

The only clear-cut biochemical abnormality demonstrated so far in these 2 patients is in the composition of the hair which has an extremely low cystine content.

Relatively little is known of the chemical structure of human hair itself, but the proteins of sheep wool are being studied intensively by several groups (reviewed by Crewther *et al.*, 1965) and the findings are probably roughly applicable to human hair. Briefly, wool proteins can be solubilized by reduction of the disulphide bridges of the cystine residues at high pH in the presence of 8M-urea, and the resultant keratines converted to their S-carboxymethyl derivatives. These may then be fractionated into high-sulphur, low-sulphur, and high-glycine proteins.

The differences in amino acid composition between the patients' hair and normal hair largely parallel the differences between hair proteins of the low-sulphur and high-sulphur groups (Table I). Thus the patients' hair appears to be deficient in high-sulphur proteins.

The cystine content of wool can be changed according to dietary conditions. Infusion of cystine into the abomasum of the sheep can increase the sulphur content of the wool from about 3 to 4% (Reis and Schinckel, 1964). This involves not only an increase in the proportion of high-sulphur protein, but also a shift within the group of high-sulphur proteins to proteins of even higher cystine content (Gillespie, Reis, and Schinckel, 1964; Gillespie and Reis, 1966). The synthesis of these high-sulphur proteins is thus subject to regulation by the availability of cystine.

There does not appear to be a serious defect in cystine transport in our patient, as the levels in the plasma and urine are normal, and there is no evidence of faulty absorption from the gut.

In sheep, copper deficiency decreases the cystine content of the wool (Burley and Horden, 1959; Gillespie, 1964), but it is not known whether this is mediated directly or by decreasing the availability of cystine by altering the gut flora. Copper deficiency in infants has been reported by Cordano, Baertl, and Graham (1964) and Cordano and Graham (1966), but there was no mention of hair changes. Zinc deficiency in animals is accompanied by hair abnormalities and parakeratosis of the skin, but human zinc deficiency has only been observed

in cases complicated by other nutritional deficiencies.

Thus, the underlying cause of the hair abnormality is obscure. It could be an isolated enzymatic defect, associated 'fortuitously' (perhaps through close chromosomal linkage) with the retardation, or there could be a common underlying biochemical disorder. A detailed chemical investigation of the hair is proceeding.

The physical nature of the hair defect invites comparison with two other inherited diseases associated with mental retardation, argininosuccinic aciduria, and the sex-linked neurodegeneration described by Menkes *et al.* (1962). In argininosuccinic aciduria there is an hereditary lack of argininosuccinase, which cleaves argininosuccinic acid to arginine and fumaric acid. Argininosuccinic acid is formed from citrulline and aspartate, and is an essential intermediate in the Krebs-Henseleit urea cycle. Hair keratin is an unusual protein in that ornithine is present in the developing hair protein, and this is then converted *in situ* through citrulline and argininosuccinic acid to arginine (Rogers, 1964). A mild degree of argininosuccinic aciduria has been reported in patients with a form of alopecia, and in one of these cases, which clearly showed trichorrhexis nodosa, argininosuccinic acid was present in the hair (Shelley and Rawnsley, 1965). A defect of argininosuccinase in the hair follicle could also account for the trichorrhexis nodosa in the case of gross argininosuccinic aciduria, but only 5 of the 11 known cases of gross argininosuccinic aciduria have the hair defect, and in one such case there was no argininosuccinic acid in the hair (Levin, 1967). It has been suggested that the absence of argininosuccinase does not apply to the whole body, since affected people can still synthesize urea.

The syndrome of sex-linked neurodegeneration and hair anomalies has a number of features not found in our cases. The disease involves progressive cerebral and cerebellar atrophy and often results in early death. Our patients are in reasonable physical health and are showing slow mental development. Menkes *et al.* reported high levels of glutamic acid in the plasma but not in CSF. Yoshida *et al.* (1964) found high levels in the CSF but only slightly raised levels in plasma, while Bray (1965) found no increase in plasma levels but did not examine the CSF. Case 1 showed no excess of glutamic acid in the plasma or CSF.

An interesting finding in this disease is that there was a deficiency of a highly unsaturated fatty acid in the brains of 2 children who died of sex-linked neurodegeneration (O'Brien and Sampson, 1966), the suggestion being that some defect permitted the

autoxidation of this substance. A fault in lipid metabolism might conceivably be linked too with the hair defect, since lipid monolayers are believed to play an important part in the orientation of the developing keratin fibres (Rothman, 1964). There was no excessive haemolysis with hydrogen peroxide (Gordon, Nitowsky, and Cornblath, 1955) in Case 1, so that a generalized defect in vitamin E metabolism can be discounted.

The hair itself is dark in our cases, whereas in sex-linked neurodegeneration the hair is unpigmented. In the cases of Menkes *et al.* the hair also showed pili torti, trichorrhexis nodosa, and irregular variations in thickness. In Bray's case the hair was of irregular thickness but trichorrhexis nodosa was not reported. Pili torti and irregular thickness was shown in the cases of Aguilar *et al.* (1966). Probably all these cases had a similar biochemical defect in the hair but with slightly different morphological manifestations. The possibility of some biochemical features in common with our cases should not be excluded, and an analysis of the hair in sex-linked neurodegeneration is awaited with interest.

Summary

A brother and sister showing mental and physical retardation and abnormal hair are described. Other members of the family were unaffected and the girl's chromosomes were normal. The hair showed trichorrhexis nodosa, a mild degree of pili torti, and a very irregular surface with partial loss of the normal scale pattern. There was an abnormality of the amino acid composition of the hair, consistent with a deficiency of the high-sulphur group of proteins.

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