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The Physicochemical Properties of Hair in the BIDS Syndrome

H. P. BADEN,[1] C. E. JACKSON,[2] L. WEISS,[2] K. JIMBOW,[1]
L. LEE,[1] J. KUBILUS,[1] R. J. M. GOLD[3]

An autosomal recessive syndrome of brittle hair, short stature, intellectual impairment, and decreased fertility in an Amish kindred has been reported [1]. Examination of the hair by scanning electronmicroscopy showed a lack of orderly scales and a defective surface with fluting and ridging. Polarization microscopy revealed an alternating birefringent pattern in all affected individuals but not in the obligate heterozygotes. The sulfur content by neutron activation analysis averaged 2.5% compared to 4.7% in normal controls and obligate heterozygotes. The hair findings were similar to those reported by Pollitt et al. [2], except in their patients, mental development and growth were more retarded. Brown et al. [3] reported a 4-year-old girl who was intellectually normal with similar microscopic and chemical abnormalities of the hair. Finally, in the Marinesco Sjögren syndrome [4] which is associated with more severe involvement of the central nervous system, alternating birefringence of the hair has been described [5].

This report presents more detailed analyses of the physicochemical properties of hair and nails in this new syndrome and relates them to structural changes observed by electronmicroscopy. Our results indicate that affected individuals have a markedly decreased amount of cystine-rich hair matrix protein.

MATERIALS AND METHODS

All the chemicals used were of reagent grade except iodoacetic acid which was crystallized from anhydrous ether and light petroleum. Hair and nails were obtained from affected individuals and their parents, stored at room temperature, washed with light petroleum, and dried under vacuum prior to being used. Hairs were plucked from some individuals and prepared for microscopy as described below.

Extraction Procedure

The specimens were extracted (10 mg/ml) in 0.2 M Tris, pH 9.5, containing 6 M urea and 0.2 M mercaptoethanol under nitrogen at 50°C for 1 hr. The mixture was

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[1] Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts.

[2] Henry Ford Hospital 2799 West Grand Boulevard, Detroit, Michigan.

[3] MRC Genetics Group, Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada.

homogenized in a ground glass homogenizer and the extraction continued for 2 hr. The suspension was centrifuged at 20,000 *g* for 30 min; the supernatant was treated with iodoacetic acid at pH 9.0 to give the S-carboxymethyl (SCM) derivative [6]. We have demonstrated that these conditions give complete reduction and blockage of ½ cystine residues. The alkylated extracts were dialyzed against distilled water, and an aliquot of the resulting suspension was dialyzed against the electrophoresis buffer and used directly for electrophoresis. The remainder of the suspension was clarified by centrifugation; the supernatant containing the matrix protein was stored at −20°C. The precipitate was redissolved in 0.05 M Tris buffer, pH 8.0, and reprecipitated at pH 4.5. After centrifugation the precipitate was washed with distilled water, dissolved in Tris buffer, pH 8.0, and stored at −20°C.

Mechanical Properties and X-ray Diffraction

The diameter of the hair was measured with an ocular micrometer after soaking the hair in water at room temperature for 30 min. One cm lengths were stretched at 0.13 cm per min in water at 25°C while recording the stress as previously described [7]. Analysis was done using nickel-filtered copper Kα radiation ($\lambda = 1.54$ Å) at 40 kV at a specimen to film distance of 1.50 cm.

Amino Acid Analysis

Samples were hydrolyzed in 6 M HCl for 24 hr under vacuum at 110°C and run on a Beckman 116 amino acid analyzer. The analyses were done in duplicate and expressed as residues per 100 residues.

Electrophoresis

Polyacrylamide disc electrophoresis followed the method of Davis [8] modified by the addition of 6 M urea. The gels were stained with coomasie blue and scanned with a Canalco microdensitometer.

Light Microscopy

The cut hairs were examined in a Vickers polarizing microscope.

Electronmicroscopy

Plucked hairs were dissected under a stereoscopic microscope into two parts based on their anatomical location: (1) within the follicular canal; and (2) protruding outside skin surface. The samples were prefixed with 2% paraformaldehyde–2% glutaraldehyde solution for 2 hrs, postfixed with 2% osmium tetroxide solution for another 2 hrs, dehydrated with ethyl alcohol solutions, and mounted in epoxy resin. The epoxy embedded tissues were sectioned with an LKB ultratone, stained with uranyl acetate and lead citrate and observed with a Siemens Elmiskop I electronmicroscope.

RESULTS

X-ray diffraction analysis of hair from three affected individuals (X-1, X-2, VIII-7) [1] showed a normal α pattern with 5.14 Å meridional and 9.8 Å equatorial reflections.

Disc polyacrylamide electrophoresis of the unfractionated SCM hair proteins gave the pattern shown in figure 1. The matrix component was markedly reduced in the five affected individuals studied (X-1, X-2, VIII-7, VIII-8, VIII-9). Although there was some variability in the amount of the matrix component, it was less than 10% of the total protein in all cases by protein determination and scan-

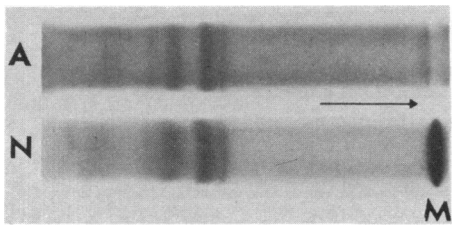

FIG. 1.—Disc polyacrylamide electrophoretic patterns of SCM-hair proteins from normal (*N*) and affected (*A*) individuals. *M* is the matrix band and the remaining bands represent the α polypeptides.

ning the gels. The α components appeared to be normal, although in certain samples from two individuals, the amount of α_1 was decreased. The yield of extracted proteins for the affected individuals was between 30% and 40% compared to about 50% for our own control specimens. Electrophoretic patterns from obligate heterozygotes were indistinguishable from normal controls.

Amino acid analyses of the hair from affected individuals are given in table 1. Although there are a number of differences from normal, the most striking and consistent one is the marked reduction in ½ cystine. The amino acid analyses of nails from two affected individuals show a marked reduction in cystine content (table 2) as well.

The stress-strain analyses of hair from affected individuals showed a striking and consistent abnormality. A typical curve is shown in figure 2. There was a decrease in the slope of the hookean and post-yield zones and a marked reduction in the stress which developed at the end of these zones. Furthermore, there was a lengthening of the yield zone. Such results were observed with six hairs from each

TABLE 1

AMINO ACID ANALYSIS OF HAIR FROM AFFECTED INDIVIDUALS

Amino Acids	X-1	X-2	VIII-7	VIII-8	VIII-9	Normal
Lysine	4.0	3.9	3.1	4.5	6.5	2.5
Histidine	1.3	0.4	0.2	0.4	0.8	0.9
Arginine	6.9	6.4	4.9	6.5	4.0	6.5
Aspartic Acid	9.1	8.1	8.8	8.4	8.9	5.4
Threonine	5.4	5.5	4.6	5.4	4.4	7.6
Serine	11.7	9.8	13.1	10.3	18.0	12.2
Glutamic Acid	14.3	14.8	16.9	15.0	13.6	12.2
Proline	5.0	5.5	3.9	5.7	5.5	8.4
Glycine	7.8	6.9	9.9	7.3	14.6	5.8
Alanine	7.3	6.6	9.8	7.1	9.8	4.3
Half Cystine	7.5	7.6	4.6	6.2	2.6	15.9
Valine	4.6	6.4	4.4	5.1	2.2	5.5
Methionine	0.6	0.6	0.6	0.7	0.5	0.5
Isoleucine	2.0	3.5	2.6	3.0	0.7	2.3
Leucine	8.4	9.4	7.8	9.5	5.0	6.1
Tyrosine	2.1	2.2	2.9	2.5	2.0	2.2
Phenylalanine	2.0	2.4	1.9	2.4	0.9	1.7

NOTE.—Results are expressed as residues per 100 residues. The patient numbers refer to reference [1].

TABLE 2

AMINO ACID ANALYSIS OF NAILS OF AFFECTED INDIVIDUALS

Amino Acids	X-1	X-2	Normal
Lysine	3.6	2.2	3.1
Histidine	1.3	0.7	1.0
Arginine	5.8	4.8	6.4
Aspartic Acid	9.3	8.4	7.0
Threonine	5.5	5.8	6.1
Serine	12.6	10.8	11.3
Glutamic Acid	17.1	17.3	13.6
Proline	5.7	7.7	5.9
Glycine	8.1	7.5	7.9
Alanine	6.6	6.3	5.5
Half Cystine	5.1	7.0	10.6
Valine	3.9	5.0	4.2
Methionine	0.5	0.6	0.7
Isoleucine	2.8	2.9	2.7
Leucine	8.8	8.8	8.3
Tyrosine	2.0	2.2	3.2
Phenylalanine	1.3	1.9	2.5

NOTE.—Results are expressed as residues per 100 residues. The patient numbers refer to reference [1].

of five affected individuals. In order to make the data clearer, control hairs with diameters identical to the abnormal ones were used for comparison. Although most hair specimens from obligate heterozygotes gave results similar to those from normal individuals, an occasional abnormal test result was observed. The abnormal

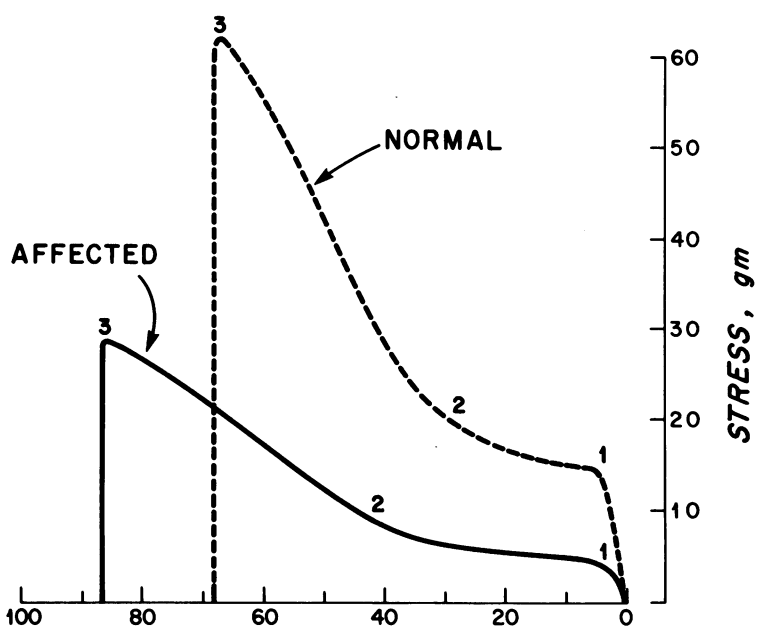

FIG. 2.—Stress-strain curves of hair from normal and affected individuals. 0–1 = the hookean zone; 1–2 = the yield zone; and 2–3 = the post-yield zone.

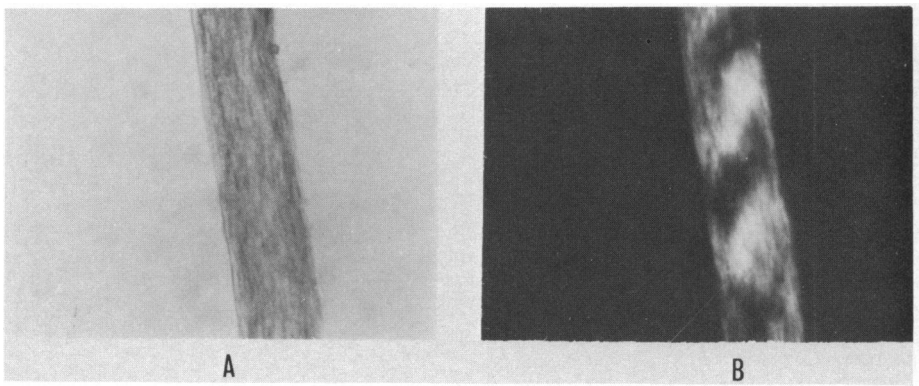

FIG. 3.—Appearance of an affected hair with regular light (*A*) and polarized light (*B*)

type of stress-strain curve could be reproduced by reducing normal hair in 0.2 M mercaptoethanol for 15 hr at 25°C.

When the hairs of affected individuals were examined in a polarizing microscope, the light was not completely extinguished at 90°C as is observed with normal hair. Instead, bright areas were seen along the shaft. When the sample was rotated a few degrees, distinct banding with light and dark areas was observed (fig. 3). The shape and width of the bands was regular for only short distances along the shaft and then changed.

Electronmicroscopic examination of plucked hairs from an affected individual showed that the most striking change was in the keratogenous zone of the hair shaft. In this zone, the keratinized cells of control specimens (hairs from an unaffected sister of the patient) revealed filamentous profiles that run parallel to the long axis of the shaft. In contrast, the keratinocytes of the subject contained filaments that ran irregularly, some crossing one another and others curling like in a whorl (fig. 4). Also, the fact that the desmosome complexes did not have a parallel orientation indicated disorganization of the cells in this region.

Light microscopic study of the hair shaft protruding from the skin showed that there was irregular staining of the cortex. At the electronmicroscopic level the cortex was seen to be composed of disorganized filament (fig. 5).

Hair samples from an individual with the Marinesco Sjögren syndrome showed a marked reduction in the amount of matrix protein by disc polyacrylamide electrophoresis which was confirmed by finding a ½ cystine content of 7.0 residues per 100 residues. Alternating bands of birefringence were observed in the hair, but an α X-ray diffraction pattern was found.

DISCUSSION

The keratinized tissues of human skin, hair, nail, and epidermis all contain fibrous proteins as major components. The appendages have, in addition, a group of cystine-rich matrix proteins which are responsible for their higher sulfur content (3%–5%) compared to stratum corneum (1%–2%) [7]. These matrix

FIG. 4.—An electronmicroscopic view of the keratogenous zone of a hair from an affected individual. Although filaments form the bundles, the bundles are disorganized and do not run parallel (× 76,000).

FIG. 5.—Electronmicrographs of hair cortex from normal (*A*) and affected (*B*) individuals. In the control specimen the filaments run in a linear array parallel to the long axis of the hair shaft; no orientation can be observed in the abnormal hair shaft (× 84,000).

proteins which contain about 30 ½ cystine residues per 100 residues are thought to act as bridges in cross-linking the filaments. This difference almost certainly explains the markedly decreased strength characteristics of stratum corneum compared to the appendages. All the data which we have presented clearly indicates that the hair of individuals affected with the syndrome has normal α fibrous proteins but a markedly reduced content of sulfur-rich matrix proteins which migrate rapidly by electrophoresis. The cystine content of hair from heterozygotes and normal controls is being measured to see if this can be used as a marker for the carrier state, although the other physicochemical characteristics have not been helpful.

The cystine values for the nails of affected individuals are low and indicate that there is a decreased amount of cystine-rich matrix proteins as in hair. These tissues are strikingly similar in their physicochemical properties, although the content of matrix proteins in normal nail is lower than in hair, and there are some minor differences in polypeptide composition [7]. Although the nails of affected individuals were thought to be normal, retrospective study revealed that they broke easily and did not grow long.

The other syndromes which have shown the nonalignment of filaments as reflected in the birefringence studies all contain lower amounts of cystine-rich matrix protein. In this study we have shown for the first time that this is also true for the Marinesco Sjögren syndrome. Since it has been found that the cystine-rich matrix proteins consist of a number of components, the defect cannot be in a single structural gene. It is possible that there has been a switch to synthesis of low sulfur matrix proteins, but no new bands were seen in the electrophoretic patterns to support this conclusion. The amino acid analyses of whole hair in affected individuals do not correspond to values reported for pure α protein, and there is some variation in the composition of different samples. This may be explained in part by the hair consisting of a number of cellular constituents. Furthermore it was not always possible to obtain specimens cut close to the scalp, and chemical alterations occur in hair exposed to the environment which affects the amino acid analysis. This is particularly true for abnormal hair.

The morphological changes which have been observed can be explained in terms of the disorganization of the filaments resulting from the absence or decreased amount of cystine-rich matrix proteins. The peculiar banding seen with polarization microscopy can best be explained by partial collapse of the hair resulting in zones of different orientation along the hair shaft. The grooving on the hair surface can be understood in terms of the internal twisting of the hair, and it is possible that the absence of cuticle cells is secondary to distortion of the hair rather than a primary defect. The appearance of the developing hair in the follicular canal suggests that the structural changes occur early and before the hair emerges from the skin surface.

Although our results do not permit a simple interpretation of the basic defect in this disease, identification of the biochemical abnormality in other affected tissues might yield a more meaningful analysis. It is of interest that a cystine-rich

keratin-like protein has been described in animal sperm [9, 10] and decreased fertility is known to be part of this syndrome. Studies of cystine-rich proteins in normal human sperm and comparisons with sperm of affected individuals could be of value, therefore, in elucidating the metabolic error.

SUMMARY

The physicochemical properties of hair from a new recessive syndrome associated with brittle hair, intellectual impairment, decreased fertility, and short stature have been studied. Electrophoresis of the SCM-structural proteins showed that the α polypeptides appeared normal, but the matrix component was markedly reduced. This was confirmed by finding a normal α X-ray diffraction pattern but a reduced ½ cystine content of hair and an abnormal stress-strain curve. Electron-microscopic studies revealed extreme disorganization of the filaments which most likely resulted from the absence of normal cross-linking. Nails, which contain structural proteins similar to hair, also showed the abnormality. Since the matrix component seen by electrophoresis consists of more than one component the defect cannot be explained as a single structural gene abnormality.

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REFERENCES

1. Jackson CE, Weiss L, Watson JHL: "Brittle" hair with short stature, intellectual impairment with decreased fertility: an autosomal recessive syndrome in an Amish kindred. *Pediatrics* 54:201–207, 1974
2. Pollitt JF, Jenner FA, Davies M: Sibs with mental and physical retardation and trichorrhexis nodosa with abnormal amino acid composition of the hair. *Arch Dis Child* 43:211–216, 1968
3. Brown AC, Belser RB, Crounse RG, Wehr RF: A congenital hair defect: trichoschisis with alternating birefringence and low sulfur content. *J Invest Dermatol* 54:496–509, 1970
4. Norwood WF Jr: The Marinesco Sjögren syndrome. *Pediatrics* 65:431–437, 1964
5. Porter PS: The genetics of human hair growth, in *The Clinical Delineations of Birth Defects*, edited by Bergsman D, Baltimore, Williams & Wilkins, 1971, pp 69–85
6. Hirs CWH: *Methods in Enzymology*. New York, Academic Press, 1967, pp 199–203
7. Baden HP, Goldsmith LA, Fleming B: A comparative study of the physicochemical properties of human keratinized tissues. *Biochim Biophys Acta* 322:269–278, 1973
8. Davis BJ: Disc electrophoresis. II. Method and application to human serum proteins. *Ann NY Acad Sci* 121:404–436, 1964
9. Bril-Peterson E, Westenbrinek HGK: A structural basic protein as a counterpart of DNA in mammalian spermatozoa. *Biochim Biophys Acta* 76:152–156, 1963
10. Hendricks DM, Mayer DT: Isolation and characterization of a basic keratin-like protein from mammalian spermatozoa. *Exp Cell Res* 40:402–412, 1969