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HEREDITARY HAIR CHANGES REVEALED BY ANALYSIS OF SINGLE HAIR FIBRES BY SCANNING ELECTRON MICROSCOPY

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Abstract

In many disorders with a genetic background the sparsity of scalp hairs may deter the clinician from trying to extract information from single hair fibres. Presenting a number of diverse conditions, we propose to show that simple measures can be taken in the doctor's office which makes single fibre analysis a useful tool for assessment of factors involved in genetic disorders including the integument and its appendages. The paper is focussed on the utilization of the scanning electron microscope with the goal of demonstrating that pertinent information can be gained where information from transmission electron microscopy and other techniques are not immediately available.

Key Words: Hair, pathology, genetic disorders, hair proteins, elemental content, scanning electron microscopy, electrophoresis, X-ray microanalysis, energy dispersive X-ray (EDX) analysis, proton induced X-ray emisssion (PIXE) analysis.

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Introduction

Leitmotif: A single event may be decisive. (Joseph E. Murray, Nobel Lecture, December 8, 1990)

Genetic disorders involving tissues of ectodermal origin are often expressed in the integument and/or its appendages. Although in recent years medical diagnosis has been greatly advanced by chromosomal analysis and immunological techniques, there is still a need for additional methods to complement the existing ones in order to obtain highest possible reliability in the diagnostic process. Hair fibres represent an interesting source of material since sampling can be performed with great ease and little, if any, discomfort to the patient. However, the amount of material available in diseased individuals is quite often minimal and analysis must consequently be performed on an odd number of single fibres. This necessitates high resolution and high sensitivity in the techniques used.

Transmission electron microscopy (TEM) represents a possible means of extracting information from single fibres. However, it involves a comparatively complicated preparation technique which demands that expert help is available in preparation, at electron microscopy and in interpretation of the micrographs (4,5). In contrast, the scanning electron microscope (SEM) offers a very simple approach to the problem with a straightforward handling of the material that can even be performed in the doctor's office (23,24). Desk top SEM instruments are now available at reasonable costs and will most likely play a role in future clinical work. The application of SEMs in the analysis of hair disorders has been recently reviewed (5,6). In the present study we sum up data on a number of cases referred to us from different parts of Sweden and discuss the feasibility of using SEM in single fibre analysis. We have, in addition, used electrophoresis and other techniques in assessing such information.

The object of the present paper is thus to demonstrate that the SEM can be a useful source of information in the analysis of diseased hairs using a straightforward and simple approach.

Material and Methods

Individuals: The 24 persons involved in this study are recorded in Table I where age and sex are given. In some instances mother and daughter-/son are represented (#2-3, #15-16). Two sisters (#17,18) a sister and a brother (#19,20) and two brothers (#21,22), a father and two sons (#22-23,24) complete the list of related individuals.

SEM: Single hair fibres were mounted on stubs carrying double-sided Scotch tape (3M) either directly without previous cleaning or after rinsing in chloroform:ethanol (1:1). It was possible to tie a knot on the fibre under a preparation microscope using watchmaker's tweezers before mounting in all but one case, where the fibre was too short (<1 cm). Most specimens were gold coated by sputtering in a Balzer's Union SCP 030 sputter unit at 5 Pa and 15 mA under continuous argon gas flow. Scanning microscopy was performed on a Philips 505 SEM instrument at 5 kV (uncoated specimens) or at 15 kV (coated specimens).

Electrophoresis: The preparation for electrophoresis was done according to Marshall and Gillespie (14,15). After chloroform:methanol (1:1) rinsing the fibres were cut into small snippets, extracted with dithiothreitol in 8M urea under nitrogen for 18 h at room temperature. Part of the aliquot was subsequently radiolabelled by S-carboxymethylation of cysteine residues by iodo-(2-14C)acetic acid in a Tris buffer, pH 8. To complete modification of cysteine residues excess iodacetic acid was added, which after 10 minutes was reacted with 2-mercaptoethanol. Until electrophoresis was done samples were stored at -20°C. Electrophoresis was run in one or two dimensions. In two-dimensional electrophoresis the first dimension was run in capillary tubes containing 8 M urea at pH 3, the second dimension on sodium dodecyl sulfate (SDS) slabs at pH 7-8. In some cases only one dimension on SDS slabs was used.

Results

The results of our investigations are summarized in Table I.

SEM: The preliminary diagnoses of trichothiodystrophy (#1,4,6,9) show a variety of appearances in the SEM ranging from very flat fibres to, on the surface, apparently normal hair strands (c.f. Figs 1, 2). To a varying extent pathological changes were revealed by the occurence of fractures through the cuticle and/or the cortex as a result of the tensions produced when a knot was tied on the fibre (Fig 3). In the straight segments such a hair may well have an apparently normal surface (#3,12). The cuticle surface appeared severely abraided (Fig 4) in many cases (c.f. #2,13). The cross section in abnormal fibres (#12) sometimes had an irregular form and even in fibres appearing normal on the surface the cortex material was loosely packed (#3,13,15,21,22) in comparison to the appearance of cross sections in normal fibres.

The two female cases, a mother and daughter with the diagnosis alopecia congenita (#15,16) both with histories of increasing hair loss (the mother, #15, wears a wig !) have flattened fibres and loose packing of the cortex material. These findings were more outspoken in the case of the mother whose fibres showed signs of trichorrhexis nodosa.

The diagnosis Netherton's syndrome (fig 5) could be established as far as concerns the hair in one case (#5). The fibres show a cross section irregularity with longitudinal spiral and shallow grooves (Fig 6) in addition to the characteristic bamboo swellings. The Haidu-Chenay syndrome cases (#7,8) showed irregular cross sections and at least one of the cases showed a subnormal cuticular adhesion to the cortex.

The BIDS syndrome case (#14) revealed an apparently low tensile strength of the cuticle as visualized by cracks formed at the knot bends (Fig 7). Also trichorrhexis nodosa phenomena were observed in the SEM (Fig 8).

The two sisters with the diagnosis epidermolysis bullosa Weber-Cockayne (#17,18) had somewhat flat fibres but revealed no apparant abnormality in the SEM.

The siblings (#19,20) with congenital psychomotoric disturbances and mental retardation had changes approximately corresponding to the severeness of their conditions. Thus the hair fibres of the sister (#19) showed a lowered cuticular adhesiveness whereas those of the brother were more normal looking.

The two brothers (#21,22) with ectodermal developmental dystrophy expressed as a dry skin prone to irritation, and no development of permanent teeth, had a dry, wiry hair. In the SEM, hair fibres were shown to be flat with a loose, compressible cortex. A further characteristic was an irregular cross section sometimes like those of pili canaliculi (glass wool hair) (Fig 9,10). In contrast, two young prepubertal sons (#23,24) of one of the two brothers (#22) had normal appearing hair fibres and were (not yet) suffering from the symptoms of their older male relatives according to oral information (given by #21).

Electrophoresis: The electrophoresis investigations have to some extent been hampered by too sparse a volume of material in a majority of the cases. Thus insufficient amounts of extracted material were at hand to provide for results allowing an unequivocal interpretation. However, in a few cases it has been possible to make a two-dimensional analysis (Fig 11). In one case (#10), the amount of hair available made it possible to perform an amino acid analysis and an abnormally low cystine content was recorded (unpublished data).

Comparison of electrophoretic patterns from mother and son (#2,3) with the tentative diagnosis of trichothiodystrophy and X-linked ichthyosis revealed differences in the one- and two-dimensional patterns. In the two-dimensional pattern from the son (#2), low sulfur, high molecular weight proteins dominated the diagrams whereas that of the mother (#3) appeared more normal in configuration (Fig 12).

It was interesting to find that the electrophoretic patterns of nail keratins appeared normal (Fig 12) even in cases where the hair keratin patterns are truly abnormal (#14).

The hair keratin of the BIDS syndrome (#14) case apparently had an abnormal high molecular weight protein pattern (Fig 12) whereas the

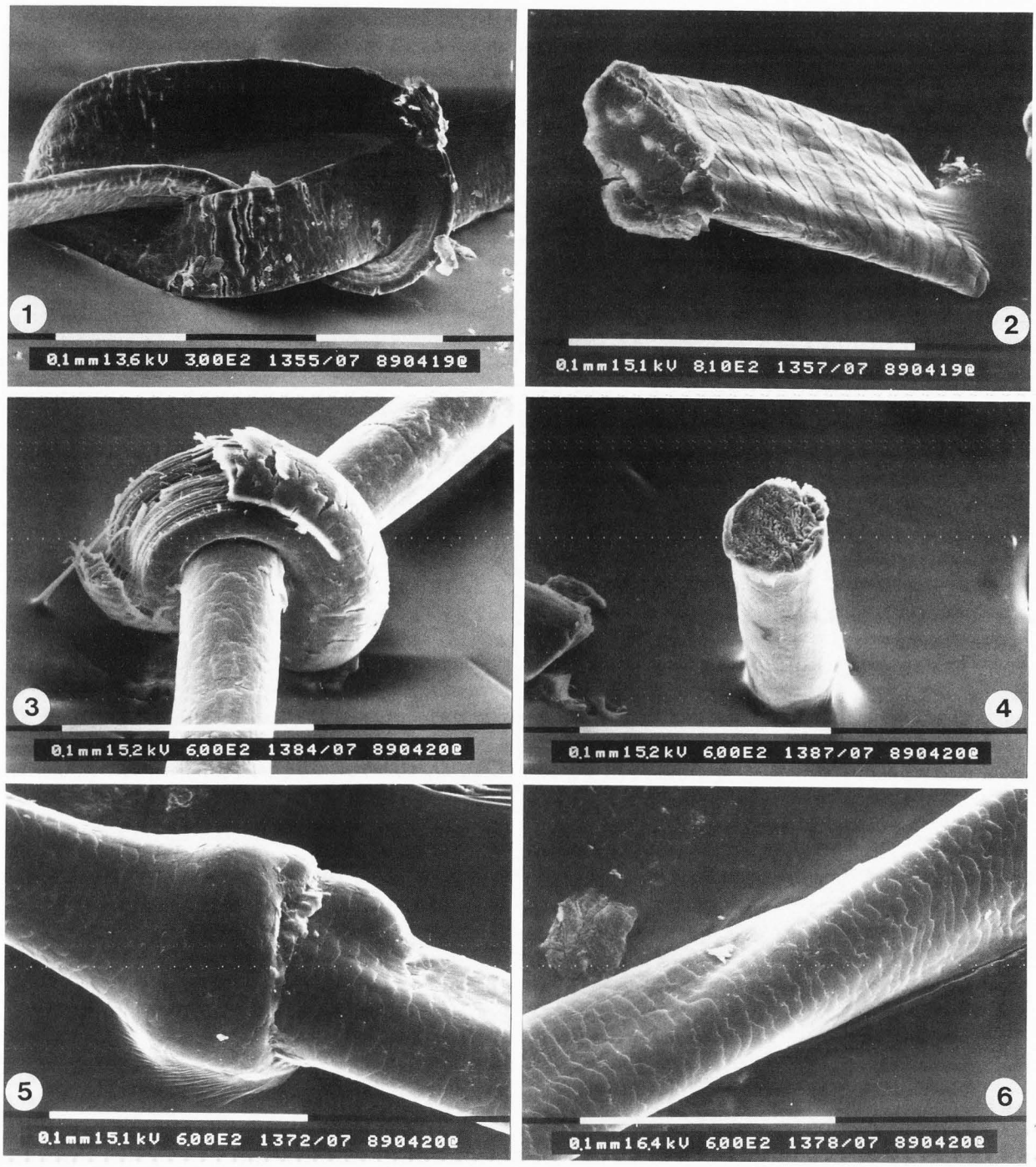


Fig 1-2. Hereditary hair disorder (case #1). Notice the cuticle is relatively intact in the straight segment (fig 2) but cracks appear as a result of the strain imposed by forming a knot (Fig 1).
Fig 3-4. Hereditary hair disorder (case # 13) Conspicuous abrasion of the cuticle is at hand. The low tensile strength of the cortex and the cuticle is demonstrated at the knot where the cortex is exposed. In Fig 4. a short segment shows the loose cortex material in a cross section.
Fig 5-6. Hair fibre from a case (#5) of Neatherton's syndrome with the characteristic bamboo thickening of the hair fibre at the point of cortex collapse. In Fig 6 a straight hair fibre segment is shown. Notice the irregular grooving which imposes a pili torti-like configuration on the fibre.

corresponding patterns of nail and skin keratin appeared normal.

Elemental analysis: Some of the cases had previously been investigated using PIXE (1) to record the elemental composition including sulfur (S), copper (Cu) and zinc (Zn) (Table I). Only in one case (#9), there was a significant low S value which also coincided with an abnormal Cu/Zn quotient. In the case given the tentative diagnosis of trichorrhexis congenita (#10), the Cu/Zn quotient was abnormally low whereas the S content appeared normal. Here the SEM findings give support for abnormality.

Discussion

The most conspicuous property of the SEM is its capacity to demonstrate the topographical relations between morphological entities of the object. The subcellular details shown in the thin sections (18) and the histochemical information possible to extract from such sections in a TEM are not available in the SEM as a routine. Concerning the analysis of hair fibres, this means that details such as a reduction in stain intensity, abnormalities in high resolution structures such as the A-band of the cuticle and identification of cell populations with a different affinity to stains used are not possible to assess in a routine SEM micrograph (10).

Few clinical cases present hair fibres that are as conspicuously kinky and abnormal as those of Menke's disease (1). The scanning electron microscope (SEM) can be used to advantage in the investigation of subtle disturbances in hair morphology. Simple preparation measures such as tying a knot on the hair fibre may give additional hints about its physical properties and may amplify defects not clearly seen on an unstrained fibre. Using a low acceleration voltage, it is possible to view hair fibres in the SEM without a previous coating of metal or carbon effected by sputtering.

The strain imposed on an abnormal hair fibre in the bends of a knot may produce cracks through the cuticle scales as well as through the cortex material. This then indicates abnormal tensile properties of the fibre. The end surface of a hair snippet cut with a razor blade, or even better, a short stub mounted on the specimen support on end, may give indication on the hair fibre cross section form (13) and on the density of the cortex material packing (8). A clinical complaint concerning hair can thus be analysed through the use of conventional SEM using simple experiments which provide opportunities for functional studies and succeeding interpretations.

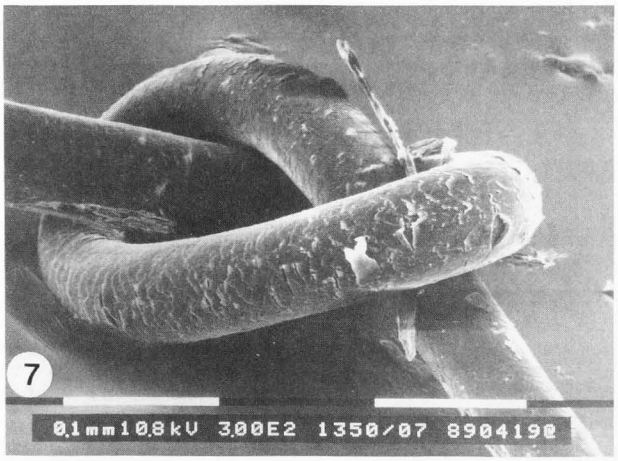
For a broader insight into the origins of diseased hair, transmission electron microscopy (TEM) provides detailed information at subcellular resolution (3,4,10,11,22,25). However, for a clinical diagnosis, information at the cellular level may well suffice and can be provided by light microscopy and SEM (24). The overviews on the structural abnormalities of the hair shaft presented by Vera Price (16,17) provide excellent introductions to the use of different microscopic techniques in the analysis of hair fibres.

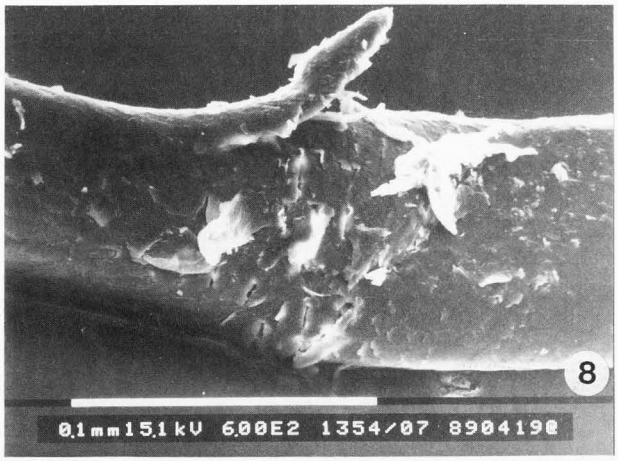
An additional technique that will complement a morphological/topographical investigation is electrophoresis. This tool which allows analysis of abnormalities in the protein fractions of hair has been used for decades. Lately, the introduction of two-dimensional electrophoresis using radiolabelled fractions from keratinized tissues has greatly improved the sensitivity and resolution of the technique (14,15). Where there are gross changes in the protein composition of the fibre, this is seen already in the one-dimensional patterns (c.f. Fig 12, #14). However, it goes without saying that extraction of protein from hair strands may vary to a great extent. In our Nordic countries, we have to accept that as little as 10-30% of the total protein content can be extracted from caucasian hair fibres of Nordic origin, whereas hair protein extractions from individuals originating in the Mediterrenean area may yield as much as 70-80% extracted material (H. Baden, personal communication 1985). In our experience, we have found that not even in severely abnormal hair does the extraction rate appear to be as much as 30%. Many of our experiments have therefore not given any conclusive electrophoretic data. In view of the detailed information that potentially can be gained with the electrophoretic technique, it is necessary to develop more efficient biochemical methods for hair fibre disintegration and extraction.

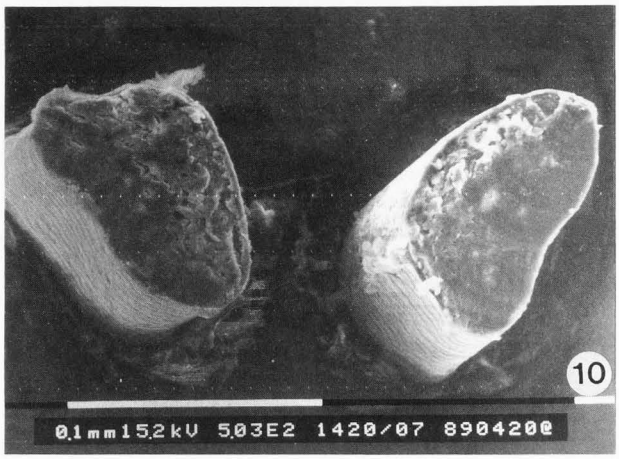
When referred to us, a number of fragile hairs were attached with a preliminary diagnosis of trichothiodystrophy. As defined by Price (17), this disorder is characterized by short, brittle and flattened fibres which may also show trichorrhexis nodosa-like fractures and an advanced weathering of the cuticle. Only cases #9 and #11 were strictly verified to be correct based on morphology, a low sulfur and low cystine values. Chemical methods for sulfur analysis require considerable amounts of hair material (12) and are therefore often not feasible when only single strands of hair material are available. In contrast, physical techniques such as proton induced X-ray emission (PIXE) analysis and energy dispersive X-ray (EDX) microanalysis allow sulfur determination from only a minute section of an intact single hair fibre (7,20,22). The technical and analytical problems involved in particle probe analysis of sulfur have been extensively discussed elsewhere (2,5,6,9). However, in the present context, the aim has been to show that, in spite of the fact that the sulfur analysis was either not at hand or alternatively not diagnostical, a SEM study may in many cases reveal hair fibre abnormalities that are indeed diagnostic.

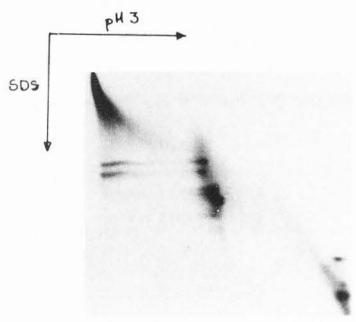
In hair analysis, the difficulty in securing a (preliminary) diagnosis at ocular inspection in the doctor's office usually means that the scientist/clinician fails to appreciate the fine structural defects which actually only can be seen at microscopic levels of resolution. The cases here presented and given the preliminary diagnosis of "short hair" (#11) and glass wool hair (#13) belong to this category.

The SEM reveals advanced structural defects suggesting that the "short hair" case (#11) should be followed up for a possible genetic background and further analysis of the elemental content including sulfur.









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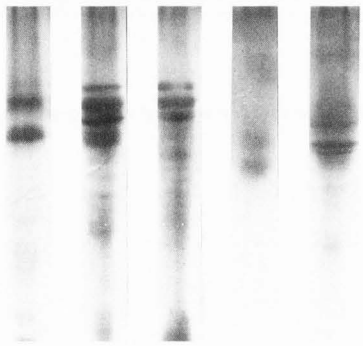

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1 2 3 4 5

Fig 7-8 Hair fibre from a case of BIDS syndrome (#14). Fine cracks through the cuticle are seen at the bends of the knot indicating a lowered tolerance to tensile stress. In Fig 8 the trichorrhexis nodosa-tendency and the conspicuous fragility of the fibre is demonstrated as a disruption of the cuticle caused by the minor trauma at the mounting for SEM.

Fig 9. A case of an unsettled diagnosis/ectodermal developmental dysplasia (#22). The knot on the fibre appears essentially normal. A groove is clearly seen at the upper bend as an indication of irregular cross section and/or loosely packed cortex material that yields under the pressure of the bend.

Fig 10. Short stubs cut from a hair of case #22. The cross section form is reminiscent of glass wool hair.

Fig 11. Two dimensional electrophoresis. First run (horisontal axis) at pH 3, second run (vertical axis) on SDS gel pH 8.

Fig 12. One-dimensional electrophoresis from case #14 (BIDS syndrome) and cases #15/16 (trichothiodystrophy, son and mother).

1st lane	case #14	hair
2nd lane	case #14	nail
3rd lane	case #14	epidermis
1st lane	case #15	hair (the son)
1st lane	case #16	hair (the mother)

A true case of "glass wool hair" will show an irregular hair cross section caused by the failure of the outer root sheath to "keratinize" , i.e. to consolidate (21). All other aspects of the keratinization process are fully normal. In contrast, case #13 turns out to have characteristics more like those of trichothiodystrophy with a severely abraided cuticle and defect packing of the cortex material.

In the analysis of hair fibre changes related to genetic disorders material from more than one member of the family involved, i.e. mother, father or siblings, is of special interest. Thus, in the SEM, the hair fibres of the mother (#3) of case #2 were superficially normal looking , whereas those of the son (#2) are extremely flat. Interestingly, in the SEM the cortex material of the hair fibres from the mother appeared more loosely packed than normal which may be interpreted as an indication of inherent abnormality.

In certain cases, the degree of changes in the hair fibres mirror the severity of the condition in general. In the two siblings (#19,20) with psychomotor disturbances and mental retardation, the severity of their hair fibre changes roughly corresponded to the severity of their neurological condition. The mother (#15) and daughter (#16) with the diagnosis Alopecia congenita showed a degree of abnormality in their hair fibre morphology which corresponded to respective clinical condition, e.g. the abnormality was most evident in the hair fibres of the mother.

The hair fibres of the two sisters (#17,18) with the diagnosis Epidermolysis bullosa Weber-Cockeyne have both essentially normal morphology as seen in the SEM.

In parallel with hair complaints, organs of ectodermal origin other than the integument, such as teeth, may be involved. In the two brothers (#21,22) with the tentative diagnosis of ectodermal developmental dystrophia, the hair growth is sparse, permanent teeth have not developed and the nails have a discoloured and rugged appearance suggesting onychomycosis. In spite of this status, numerous cultures to secure a fungus diagnosis have proved negative. The hair fibre morphology as seen in the SEM suggest abnormality of the cortex material. There is a conspicuously loose packing of cortex substance, longitudinal grooves on the fibre surface and indications of compressible cortex at knot bends. Such features are to a lesser extent also seen in the sons of one of the two brothers (#23,24).

The present paper has demonstrated the feasibility of an experimental approach to hair analysis performed in the SEM, e.g. tying a knot, cutting short stubs that can be seen end-on etc, in addition to the conventional morphological (and subjective) analysis by ocular inspection of SEM micrographs. Thus, taking full advantage of the SEM investigations of single fibres from hair disorders may provide clues for a good diagnosis. The advantage of using complementary techniques such as transmission electron microscopy and electrophoresis in one or two dimensions, physical techniques for elemental analysis, e.g. S, Cu, Zn, for establishing a correct diagnosis in systemic and integumental disorders reflected in hair abnormalities is underlined.

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References

1. Collie WR, Goka TJ, Moore CM, Howe RR (1980) Hair in Menke's diease: A comprehensive review. In AC Brown, RG Crounse (eds) Hair, trace elements and human illness. Praeger Scientific, New York, pp 197-209.

2. Eatough DJ, Christensen JJ, Izatt RM, Hartley C (1974) Levels of selected trace elements in human hair. Chapt 32 in AC Brown (ed), The First Human Hair Symposium, Medcom Press, New York, pp 377-387.

3. Forslind B (1979) Die wachsende Anagenphase. In CE Orfanos (ed) Haar und Haarkrankheiten. Gustav Fischer Verlag, Stuttgart, New York, pp. 51-75.

4. Forslind B (1990) The growing anagen hair. In CE Orfanos, R Happle (eds) Hair and hair diseases. Springer Verlag, Berlin, New York, London, Paris, Tokyo, pp 73-97.

5. Forslind B (1984) Clinical applications of scanning electron microscopy and X-ray microanalysis in dermatology. Scanning Electron Microsc/1984/I:183-206.

6. Forslind B (1988) Clinical applications of scanning electron microscopy and energy dispersive X-ray analysis in dermatology - An up-date. Scanning Microsc 2:959-976.

7. Forslind B, Li HK, Malmqvist KG, Wiegleb, D (1986) Elemental content of anagen hairs in a normal caucasian population studies with proton induced X-ray emission (PIXE). Scanning Electron Microsc/1986/I:237-241.

8. Forslind B, Wiren K, Malmqvist KG (1987) Assessment of qualitative and quantitative data from pathological hairs - A critical evaluation of scanning electron microscope and proton induced X-ray emission analyses. Scanning Microsc 1:159-167.

9. Forslind B, Malmqvist KG, Wiren K (1988) Genetic diaseases, hair structure and elemental content. Chapt 19 in GE Rogers, PJ Reis, KA Ward, RC Marshall (eds) The Biology of Wool and Hair, Chapman & Hall, London & New York, pp 275-285.

10. Gummer CL, Dawber RPR, Price V (1984) Trichothiodystrophy: an electron histochemical study of the hair shaft. Brit J Dermatol 110:439-449.

11. Gummer CL, Dawber RPR, (1985) Trichothiodystrophy: an ultratructural study of the hair follicle. Brit J Dermatol 113:273-280.

12. Kutner M, Miller K, Brown AC (1974) A critique: Hair sulfur analysis for evaluation of normal and abnormal hair. In AC Brown (ed) The First Human Hair Symposium, Medcom Press, New York, pp 363-376.

13. Lindelöf B, Forslind B, Hedblad M-A, Kaveus U (1988) Human hair form. Morphology revealed by light and scanning electron microscopy

Table I: Survey of cases examined

Patient	Age yrs.	Sex	Preliminary diagnosis	Scanning Electron Microscopy findings	Electrophoresis	PIXE %S	PIXE Cu/Zn
1	1	M	Tay syndrome Trichothiodystrophy ichtyosis	Flat fibres, low tensile strength of cuticle, cortex volume small	High MW proteins appear normal		
2	21	M	Trichothiodystrophy X-linked ichtyosis	Abraded cuticle, trichorrhexis nodosa, low adhesiveness of cortex	High MW proteins appear normal		
3	47	F	Trichothiodystrophy Follicular hyperkeratosis	Normal appearance on surface; cortex material loosely packed, compressible	High MW proteins appear normal		
4	3	F	Trichothiodystrophy "collodium baby"	Flat fibres, low tensile strength of cuticle, cortex volume small; trichorrhexis nodosa, ripples on surface and short longitudinal grooves	?		
5	3	F	Netherton's syndrome trichorrhexis invaginata, nodular ichtyosis	Cuticular pattern normal, pili torti, bamboo hair, loose cortex	?		
6	10	F	Trichothiodystrophy	Normal surface appearance	?		
7	9	M	Haidu-Chenay syndrome	Cuticle edge-to-edge distance short, irregular cross-section			
8	17	M	Haidu-Chenay syndrome	Subnormal adhesion of cuticle cells, irregular cross-section	?	6.4	0.11
9	1	M	Trichothiodystrophy	Extremely flat fibres, short longitudinal grooves, abraded cuticle	?	5.1	0.17
10	7	F	Trichorrhexis congenita	Cuticle adhesion weak, fibres look like thin-walled tubes, some fibres break easily at knot	?	2.1	0.06
11	2	F	"Short hair"	Pili torti, spontaneous breaks, low tensile strength	Amino acid analysis: low cystine	4.6	0.08
12	6	F	Trichothiodystrophy	Some fibres normal, some irregular cross-sections	?		
13	4	F	Glass wool hair	Abraded cuticle, low tensile strength loosely packed cortex material	?		
14	?	M	BIDS syndrome lamellar ichtyosis	low tensile strength of cuticle, tendency to trichorrhexis nodosa, cuticle easily abraded	Abnormal high MW proteins		
15	15	F	Alopecia congenita	Flattened fibres in some loose cortex material, irregular cross-section, longitudinal grooving	?		
16	41	F	Alopecia congenita	Flattened fibres, low adhesiveness of cuticle, tendency to trichorrhexis nodosa	?		
17	?	F	Epidermolysis bullosa Wbeber-Cockeyne	No conspicuous abnormality, fibres somewhat flat	?	6.2	0.63
18	?	F	Epidermolysis bullosa Wbeber-Cockeyne	No conspicuous abnormality, fibres somewhat flat	?	5.9	0.41
19	13	F	Psychomotor retardation (severe)	Cuticle adhesiveness appears low, round cross-section	?		
20	10	M	Psychomotor retardation (mild)	Normal cuticle configuration, oval cross-section	?		
21	?	M	Ectodermal developmental dystrophy	Flat fibres, loose, compressible cortex material	?		
22	?	M	Ectodermal developmental dystrophy	Longitudinal grooving, irregular cross-section like pili canaliculi	?		
23	?	M	Ectodermal developmental dystrophy	Longitudinal grooving, irregular cross-section like pili canaliculi	?		
24	?	M	Ectodermal developmental dystrophy	Longitudinal grooving, irregular cross-section like pili canaliculi	?		

and computer aided three-dimensional reconstruction. Arch Dermatol 124:1359-1363.

14. Marshall RC, Gillespie JM (1982) Comparison of samples of human hair by two-dimensional electrophoresis. J Forensic Science Soc 22:377-385.

15. Marshall RC, Gillespie JM, Klement V (1985) Methods and future prospects for forensic identification of hairs by electrophoresis. J Forensic Science Soc 25:57-66.

16. Price, VH (1979) Structuranomalien des Haarschaftes. In CE Orfanos (ed) Haar und Haarkrankheiten. Gustav Fischer Verlag, Stuttgart, New York, pp.387-446.

17. Price, VH (1990) Structural anomalies of the hair shaft. In CE Orfanos, R Happle (eds) Hair and hair diseases. Springer Verlag, Berlin, New York, London, Paris, Tokyo, pp. 363-422.

18. Rogers, GE (1959) Electron microscopy of the hair folllicle J Ultrastruct Res 2:309-330.

19. Salinas CF, Spector M (1980) Tricho-dental syndrome. In AC Brown, RG Crounse (eds) Hair, trace elements and human illness. Praeger Scientific, New York, pp 240-256.

20. Seta S, Sato H, Yoshino Y, Miyasaka S. (1982) SEM/EDX analysis of inorganic elements in human scalp hairs with special reference to the variation with different locations on the head. Scanning Electron Microsc/1982/I:127-140.

21. Stroud JD (1980) Complementation of the inner root sheath of human hair. In AC Brown, RG Crounse (eds) Hair, trace elements and human illness. Praeger Scientific, New York, pp 163-168.

22. Swift JA (1981) The hair surface, in CE Orfanos, W Montagna, G Stuttgen: Hair research, Springer Verlag, Berlin, Heidelberg, New York, pp. 65-72.

23. Van Neste D, Lachapelle JM, Antoine JL (1989) Trends in Human hair growth and alopecia research. Kluwer Academic Publishers, Dordrecht, Boston, London. pp 1-330.

24. Van Neste D, Hubion Y (1986) Office diagnosis of changes in hair cuticular cell patterns. Arch Dermatol 122:750-752.

25. Venning VA, Dawber RPR, Ferguson DJP, Kanan MW (1986) Weathering of hair in trichothiodystrophy. Brit J Dermatol 114:591-595.

Discussion with Reviewers

JA Swift: How are you able to knot a hair using watchmaker's tweezers without damaging the surface of the fibre ?
Authors: By using a segment of the fibre, 1-2 cm long, and only handling the fibre at the ends, it is with a little experience possible to form a knot. Actually, if marks of the tweezers appear on the fibre surface this may indeed indicate decreased mechanical properties of the fibre. A normal hair fibre will generally suffer minute cuticle abrasion, appearing as chipping, at the contact area of the instruments.

JA Swift: In figures 1, 3 and 7, the tightness of the knots seems to vary. Does this not give rise to difficulties of comparative interpretation ? In this respect the delamination of the cortex (and not as stated the cortex) seen in Fig 3 is entirely consistent with what this questioner has observed in perfectly normal hair held tightly with a small radius of curvature (27)
Authors: Obviously the tightness of the knot is influenced by the pulling force when forming the knot, the tensile properties of the fibre, and the fibre diameter. When tying a knot manually it is difficult to have a full control of the pulling force. It is my experience (BF), however, that normal hair fibres seldom allow a very tight knot whereas on pathological fibres a tight knot is easily produced. Thus even the tightness of the knot may to a certain extent give information on the mechanichal properties of the fibre.

Concerning delamination, I (BF) have never observed this in normal hair fibres except in segments subject to severe weathering, i.e. at distances more than 10 cm from the scalp. Consequently we use only the most proximal part of the fibre. When possible, our clinical colleagues are encouraged to pull the hair samples to obtain samples including the root and a virgin part of the hair fibre for the same reason.

JA Swift: Given the diversion and often overlapping expression of abnormal hairs in terms of their surface appearance in the SEM, do you believe that the protein analytical methods will turn out to be more reliable diagnostically than the SEM appearance of the hairs ?
Authors: Since the evaluation of a SEM micrograph is a matter of experience and therefore to a certain extent subjective, we believe that the firmest answers are given by protein analysis. At present only few clinicians have the laboratory service available for such analyses and therefore the SEM will provide a next to the best answer to the question if a fibre is pathologically changed. In the ideal case the whole arsenal of biochemical and biophysical techniques are employed. However, we also believe that there is still a great need for base data obtained from cases where a diagnosis has been unambiguously stated. A future task is therefore to compile the information given in case reports like the present one.

Reviewer II: Is there any worn hair but not a pathological one revealing fractures in the same knotting examination ?
Authors: So far; we have no experience with such cases.

Reviewer II: What is the abnormal electrophoretic pattern in scalp hairs ?.
Authors: Abnormality is deduced from the deviations in patterns when compared to those of normal hair (cf ref 14,15)

Reviewer II: What is the range of Cu/Zn quotient value for healthy scalp hairs ?
Authors: At present we believe it to be 0.05-0.27 as related to the quantitative data given in ppm (9). This range is deduced from the data given by Iyengar et al. (28) is shown in Table II. However, the wide range of data for Cu and Zn which depends on the method of analysis and the mode of sampling makes this range somewhat uncertain. We are there-

fore in the process of setting up an investigation of hair from one hundred normal individuals using an X-ray fluorescence method developed at Chalmers' Institute of Technology, Gothenburg and the Forensic Research institute of Sweden (Stocklassa et al. unpublished data) which will hopefully be published during the year to come.

EB Rest: How much variability exists between hair shafts in each and any disorder ? What is the minimum number of hair shafts necessary to accurately diagnose a structural abnormality ? Are findings from one or two hair shafts applicable to the majority of hair shafts ?
Authors: You are certainly touching upon a sore point in single hair fibre analysis. A natural scientist will demand that a statistically well founded evidence requires >20 fibres for an analysis and a sound decision of the level of significance required for an abnormality to be present. In the practical situation such an analysis is impossible due to lack of material, time and money. Thus, the clinician has to rely on the case history and isolated fibres chosen for their "suspicious" look. The total number of hair fibres you study in the SEM and with EDX-methods are thus related to the need for a precise diagnosis.

In short: diagnosis is seldom based solely on biophysical data from SEM, TEM, and EDX. Clinical evaluation of case history etc plays an important role as well.

EB Rest: There are overlapping features of hair shaft anomalies, i.e. longitudinal grooving is seen in a number of disorders. Which features do the authors consider most helpful in establishing a diagnosis with a small number of hairs ? If this technique is intended for "routine" clinical use, perhaps a table of the most diagnostic features would be helpful.
Authors: Cuticle appearence, e.g. abnormal chipping or abrasion and "spontaneous", alternatively induced complete or partial fractures are the most conspicuous features you look for in the SEM. Rather than to provide the desired table we have to refer to ref. 17.

EB Rest: What is the role of scalp biopsy in diagnosis of hair shaft defects. Is the scalp biopsy (horizontal sections) helpful with SEM ?
Authors: Scalp biopsies are valuable when disorders of the inner and outer root sheath may be suspected, e.g. "spun glass hair". Also, in conditions which may have an immunological background such as Alopecia areata where immunological methods may prove diagnostical (29).

GM Roomans: You mention the BIDS syndrome (#14). How is this acronym defined ?
Authors: Not even in Baden's original paper from 1976 (26) is is the acronym BIDS strictly defined. It is a syndrom in Amish kindred with brittle hair (B), intellectual imparement (I), decreased fertility (D) and sulfur deficiency (S) of the hair which has weakened tensile properties.

Additional references:

26. Baden H, Jackson CE, Weiss L, Jimbow L, Lee L, Kubilus J, Gold RJM (1978) The physicochemical properties of hairs in the BIDS syndrome. Am J Hum Genet 28:514-521.
27. Brown AC, Swift JA (1975) Hair breakage - the scanning electron microscope as a diagnostic tool. J Soc Cosmet Chem 26:289-297).
28. Iyengar GV, Kollmer WE, Bowen HJM (1978) The elemental composition of human tissues and body fluids. A compilation of values for adults. Verlag Chemie, Weinheim, New York 1978 pp 51-54)
29. Perret C, Happle R (1990) Treatment of Alopecia areata. In Orfanos CE, Happle R (Ed.s) Hair and hair diseases. Springer Verlag, Berlin, New York, London, Paris, Tokyo, pp. 571-586.

Table II The great variation of data on the elemental content of "normal" hair. Data compiled from Iyengar et al. (28)

ELEMENT	CONTENT x 10E-6	MAIN METHOD USED
As	0.13 - 3.71	NAA
Au	0.0017 - 1.25	NAA
Br	0.65 - 53.3	NAA
Ca	146 - 3190	NAA/AAS
Cd	0.24 - 2.7	AAS
Cl	950 - 4805	NAA
Co	0.2 - 1.05	NAA
Cr	0.13 - 3.65	NAA/AAS
Cu	11 - 34	AAS/NAA
Fe	5 - 44.7	AAS/NAA
Hg	1.25 - 7.6	NAA/AAS
I	0.085 - 15.1	NAA
K	150 - 663	AAS/FES
Mg	19 - 163	AAS
Mn	0.25 - 5.7	NAA
Mo	0.064 - 2.83	NAA
N	140 - 157	CAT/XRF
Na	18 - 1720	NAA
Ni	0.6 - 6.5	AAS/NAA
P	83 - 165	SAS
Pb	3 - 70	AAS
S	695 - 47.10E-3	SAS
Sb	0.09 - 3	NAA
Se	0.64 - 2.53	NAA
Sn	1	MS
Sr	0.15 - 14.7	
Tl	0.016 - <1.8	NAA/MS
U	0.00013	
Zn	99. - 450	AAS/NAA

AAS: atomic absorption spectroscopy
AES: arc emission spectroscopy
CAT: catalytic methods
FES: flame emission spectrometry
MS: mass spectroscopy
NAA: neutron activation analysis
SAS: solution absorption spectrometry
XRF: X-ray fluorescence spectrometry