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THE USE OF SCANNING ELECTRON MICROSCOPY IN THE ANALYSIS OF PATHOLOGICAL HAIRS

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Abstract

The potentials of the scanning electron microscope (SEM) have only to a certain degree been exploited in the study of pathological hair fibers. In this review brief viewpoints on aspects of preparation and interpretation are discussed. It is shown that SEM will reveal important facts on the morphology of pathological hairs is appropriate experiment are performed, such as forming a knot on the fiber. Such a simple experiment will provide information on the fiber cross section, and on the tensile strength of the cuticle and the fiber. Complementary methods for qualitative and quantitative analysis of pathological hair fibers are suggested.

Key words: Hair, normal, pathological, scanning electron microscopy

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Introduction

Scanning electron microscope (SEM) analysis of pathological hairs was one of the first and a prominent application when pathologist first began this instrument. Undoubtedly this was due to the ease by which hair samples could be prepared for scanning microscopy. This is of course due to the fact that the hair fiber is a specimen which is already extensively dehydrated as it emerges out of the follicle. Also, the proteins of hair fibers are (relatively) stable in the electron beam. Thus this type of specimen can be subjected to prolonged scrutiny in the SEM.

The SEM has played an important role in the development of hair pathology. An early source of inspiration was the **"First Human Hair symposium"** in Atlanta (1) in 1973 followed by **"The First International Hair Congress"** in Hamburg, West-Germany in 1978 (12). This year (1989) the formation of a **European Hair Research Society** indicates the increasing importance of and interest in hair research.

A.C. Brown published a review on SEM as applied to the integument (3), the main part of which was devoted to studies on hair and this review covers the literature before 1979. Under the heading of "The Integument" a selection of papers published during the years 1978-1985 in Scanning Electron Microscopy comprise the application of SEM and X-ray microanalytical techniques as applied to clinical and experimental dermatology (6). Included in this volume is a review on SEM as applied to dermatological problems covering the years 1969-1983 (5). More recently a sequel review has been published on the clinical applications of SEM and energy dispersive X-ray microanalysis covering the literature up to 1987 (8).

The object of this paper is to highlight some important aspects of the application of SEM to hair analysis and to convey some simple hints which allow extraction of extra information about hair fiber properties. It is hoped that this will be an inspiration to further the use of the scanning electron microscope in clinical, pathological and experimental medicine.

Preparation of hair fibres for SEM analysis

Cleansing procedures: Hair fibers may be mounted for SEM without previous cleansing procedures. More often than not debris originating from the environment, including the scalp, will adhere to the fiber surface. Since the lipids from the sebaceous glands form a thin film covering the surface of the fiber lipid solvents often free the fibers from fat and debris. It appears that the best solvent combination is chloroform:methanol (1:1) which can be followed by a brief rinse in distilled water if salt deposits are present.

Fixation: Usually hair fibers are viewed in the SEM without prior fixation. It has been argued that fixation by chemical means or by critical point drying is necessary for stabilization of fibers before the introduction into the SEM (9). A critical analysis of the arguments presented in the literature reveal that no satisfactory control experiments to support such a claim have been presented. Since keratin is a highly hygroscopic material any fixation experiment should include a rigid control of the hydration parameter during the **entire** preparation procedures to account for changes in the hair fiber form and/or diameter as well as in the internal fibrillar and cellular architechture. The conclusion is that from a practical point of view an additional fixation step does not seem necessary when consolidated fibers are studied.

Specimen mounting: Mounting the fiber on a SEM stub can be done using fluid carbon or a glue. Alternatively double sticking tape provides a convenient mounting medium. The fluid mounting materials have the drawback that the cuticle cells of the hair fiber surface and exposed parts of cortex may draw liquid. This will make the fiber partially immersed in the mounting material and consequently some areas of the fiber will not be accessable to inspection. Especially the part-time microscopist will find double sticking tape an easier and more reliable mounting material, albeit it is more difficult to keep the background surface dust free.

Coating the specimen: Dry protein materials, like a hair fiber, are good electric insulators and the cleaned fiber will often have electrostatic properties. This will result in charging spots on the specimens under electron bombardment. It is therefore necessary to coat the specimen with a conducting coat. Before coating the hair fiber the specimens mount sandwich should preferably be cleaned with a spray-type duster. The free tips of the cuticle cells are often conspicuous sites of charging. Evaporating a thin film of gold (Au), gold/platinum (Au/Pt) alloy or carbon on the surface of the fiber in vacuum provides the specimen with the desired conducting layer. In addition, a metal coating will provide an effective stopping material for the impinging electrons on the very surface of the biological structure within the normal range of acceleration voltages (5-20 kV). The deposition of metal or carbon on the surface can be achieved with a moderate vacuum sputter or a high vacuum evaporator. Generally sputtering will be satisfactory for the resolution desired. However, in high resolution studies high vacuum evaporation is mandatory. In addition, minute surface structures which may be hidden under a sputter coat can be given an enhanced contrast in the final image by unidirectional low angle evaporation of coating material.

Electron microscopy: Uncoated or coated speciments are studied to advantage at low voltage, e.g. 5 kV, if high resolution images are not required, i.e. if the primary magnification is below 2000 X. Charging at the cuticle free ends is then generally avoided. For higher resolution 15 kV is generally used.

Interpretation and experimental approach

In the introductory chapter of the publication from "The first human hair symposium" Brown outlined the feasibility of hair analysis in the SEM (2). As main characteristics to be looked for he directed the interest towards **the cuticle** which may be present or absent, a fact often correlated to tensile strength and also to content of cystine and thus sulfur. Instead of the smoothly straight or slightly undulating free border of the virgin fiber (Fig. 1) in the 'normal' process of weathering, i.e. the effect of physical and chemical wear and tear on the fiber, little bits of the cuticle free end are chipped away, leaving a jagged contour (Fig. 2). The size of the chips broken away from the fiber is likely to be correlated to the adhesiveness of the inter-cuticle/cuticle-cortex cementing substance. Other interesting cuticle characteristics are the distance between the free edges of overlapping cuticle cell, cuticle cell thickness, and the number of cuticle cells enclosing the fiber circumference. The cuticle surface may be smooth or corrugated by fine longitudinal ridges. In addition to the mentioned variations in the cuticle morphology related to the intrinsic properties of a hair fiber chemical and physical agents alone or in combination may produce a variety of artifactual changes as seen in SEM micrographs.

At the tip end of a scalp hair fiber the cuticle cells often are only partly present and the cortex is laid bare (Fig. 3). Fringing of the cortex may be conspicuous in fibers that have been exposed to extensive weathering. The degree to which this is seen appears to be related to individual properties of the hair shaft, the extent to which the fiber has been subjected to cosmetic treatment, other environmental factors etc. Thus, this finding should be interpreted with utmost care. To minimize misinterpretation **the virgin part of the fiber**, e.g. the part closest to the scalp should always be included in the investigation.

The hair fiber cross-section may be easily visualized by cutting a millimeter long stub from the fiber fixed on the SEM stub by double sticking tape. Gentle manipulating of the cut piece to make it stand on one end will allow easy inspection of the cut cross section in the SEM. In a defect fiber compression effects in the cortex material at the cut end will often be more conspicuous than in normal hair shafts (Fig. 4).

The scalp hair shaft normally appears as a cylinder or a slightly compressed cylinder in the

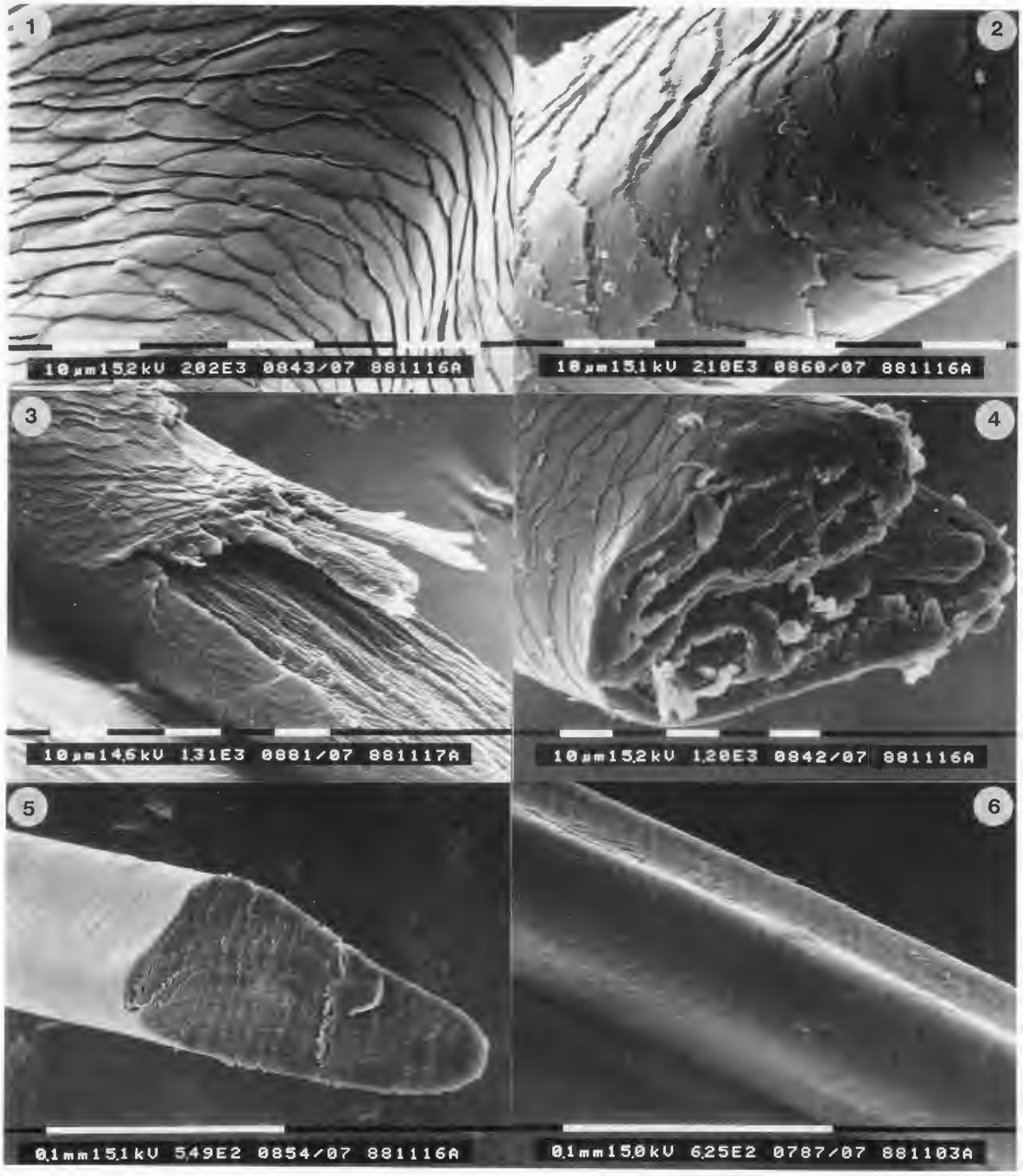

Fig 1. Virgin hair fiber. Notice the smooth free ends of the cuticle cells. White bar 10 µm.

Fig 2. Weathered fiber. The free edges of the cuticle scales are chipped and the cell margin is dagged from the chipping. At places the cuticle is lifted up from the underlying surface. White bar 10 µm.

Fig 3. Free end of weathered fiber with cortex laid bare. In the lower part of this micrograph the cuticle cells are severely abraded. White bar 10 µm.

Fig 4. Short end of a hair fiber showing a pathologically loose structure of cortex material. White bar 10 µm.

Fig 5. Cross section of a pubic hair fiber. Notice the kidney form which is similar to that often seen in pili canaliculi. White bar 100 µm.

Fig 6. Pubic hair with longitudinal groove. White bar 100 µm.

SEM. It may also disclose longitudinal groove(s) which generally are shallow. In pubic (Fig. 5 & 6) and axillary hair this longitudinal grooving is a generalized finding and a characteristic of forensic interest. Also in certain conditions such as in **pili canaliculi** longitudinal grooves run along the fiber just for short distances. Sequential cuts of such a hair fiber will show that the cut end of the fiber sections from one and the same hair shaft appear to have different shapes, e.g. kidney, crescent or oval form.

The fiber diameter may vary as a function of disease referred to as the Pohl-Pinkus mark. The phenomenon has been observed in diseases related to protein synthesis deficiencies, for example in Kwashiorkor. It may also be an iatrogenic effect caused by drugs such as used in cancer therapy. Drugs directed towards control of nuclec acid synthesis (4) fall within this category. The effect of such substances should be correlated to findings of the number of anagen/telogen hair fibers. It is, in this context, important to observe that the hair fiber diameter becomes reduced in conditions of **protein deficiency** whereas **caloric deficiency** results in an increased number of telogen hairs (30%).

The effect of a **sulfur deficiency** is often seen as clear breaks in the fiber, trichorhexis nodosa (Fig. 7), or bamboo type of invaginations of the fiber (Fig. 8) in addition to the cuticle observations indicated above.

The tensile strength of a hair fiber can be investigated by simply **tying a knot in the fiber** (7). If the cuticle cementing substance is defective the cells tend to stand out radially in the sharp bends of the knot (Fig. 9). Minor breaks in the cuticle cell surface indicate an internal deficiency in the cuticle fibrillar and/or matrix substance. In more severe cases of deficiencies in the protein synthesis the fiber will break partially (Fig 10) or completely at the knot.

The knot also reveals the cross-sectional form of the fiber. The sharp bends of the fiber in the knot appear flat in an oval fiber but are clearly rounded in a hair shaft with a more or less circular cross-section (Fig. 11). If the packing of cells and/or intracellular material in the center of the fiber is conspicuously loose or if the medulla is prominent a tight knot will show a shallow groove in the bend profile (Fig. 12).

Complementary techniques

Although the SEM can be used to record the morphology of and to disclose a number of properties of the scalp hair shaft, often complementary information is needed for a clinical/pathological diagnosis. Today **energy dispersive X-ray microanalysis (EDX)** in the SEM provides information on the elemental composition of the fiber under investigation. Sulfur which is difficult to determine biochemically is conveniently determined with this technique. The main drawback of the EDX is that it gives information only on the chemical composition and not on the chemical state of the elements, e.g. EDX does not show if the elements are in ionized form, or if they are bound in certain chemical configurations etc. In inertly prepared specimens the sensitivity of EDX is approximately 200 ppm (parts per million). If there is a need for trace element analysis the sensitivity can be increased by microincineration of the specimens or by proton induced X-ray emission (PIXE) analysis (8). The former technique may increase the sensitivity by a factor of 2-5 whereas the latter method at present allows a sensitivity of <10 ppm (8).

Biochemical analysis, including amino acid analysis, may be quite rewarding. Often the amount of material available will prohibit amino acid analysis. Using radiolabelling the fractions of dissolved hair proteins can be subjected to two-dimensional electrophoresis starting from minute amounts of material, i.e. a 5 mm strand of hair (11).

Looking at the research in progress in the wool industry, it may be noted that a number of staining techniques for analysis of wool fiber characteristics are evolving (10). It is most likely that such techniques will provide pertinent information to pathologists, forensic scientists and dermatologists alike concerning the properties of hair fibers from normal and pathological cases.

Conclusion

Although the SEM by now is a firmly established technique in dermatological pathology it is quite often overlooked by dermatologists in the analysis of hair shaft abnormalities. The suggestions for a functional approach given here as a complement to pure morphological recording of the hair fiber surface will hopefully invite a new interest in the SEM as a tool for hair fiber analysis.

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References

1. **Brown AC, ed. (1974)**. The First Human Hair Symposium. Medcom Press, New York.
2. **Brown AC (1974)**. Introduction: Blood, urine and hair. In: AC Brown (Ed.) The First Human Hair Symposium. Medcom Press, New York, pp 1-24.
3. **Brown AC (1980)**. The integumentary system. In: GM Hodges and RC Hallowe (Eds.) Biomedical Reseacrch applications of scanning electron microscopy. Academic Press. London, pp 65-125.
4. **Crounse RG, Van Scott EJ (1960)**. Changes in scalp hair roots as a measure of toxicity from cancer chemotherapeutic drugs. J Invest Dermatol 35: 83-90.
5. **Forslind B (1984)**. Clinical applications of scanning electron microscopy and X-ray microanalysis in dermatology. Scanning Electron Microscopy 1984/I/183-206.
6. **Forslind B, Kischer CW, Roomans GM** (Eds) **(1985)**. The Integument. Scanning electron microscopy in skin biology. Scanning Electron Microscopy Inc. AMF O'Hare, Chicago.

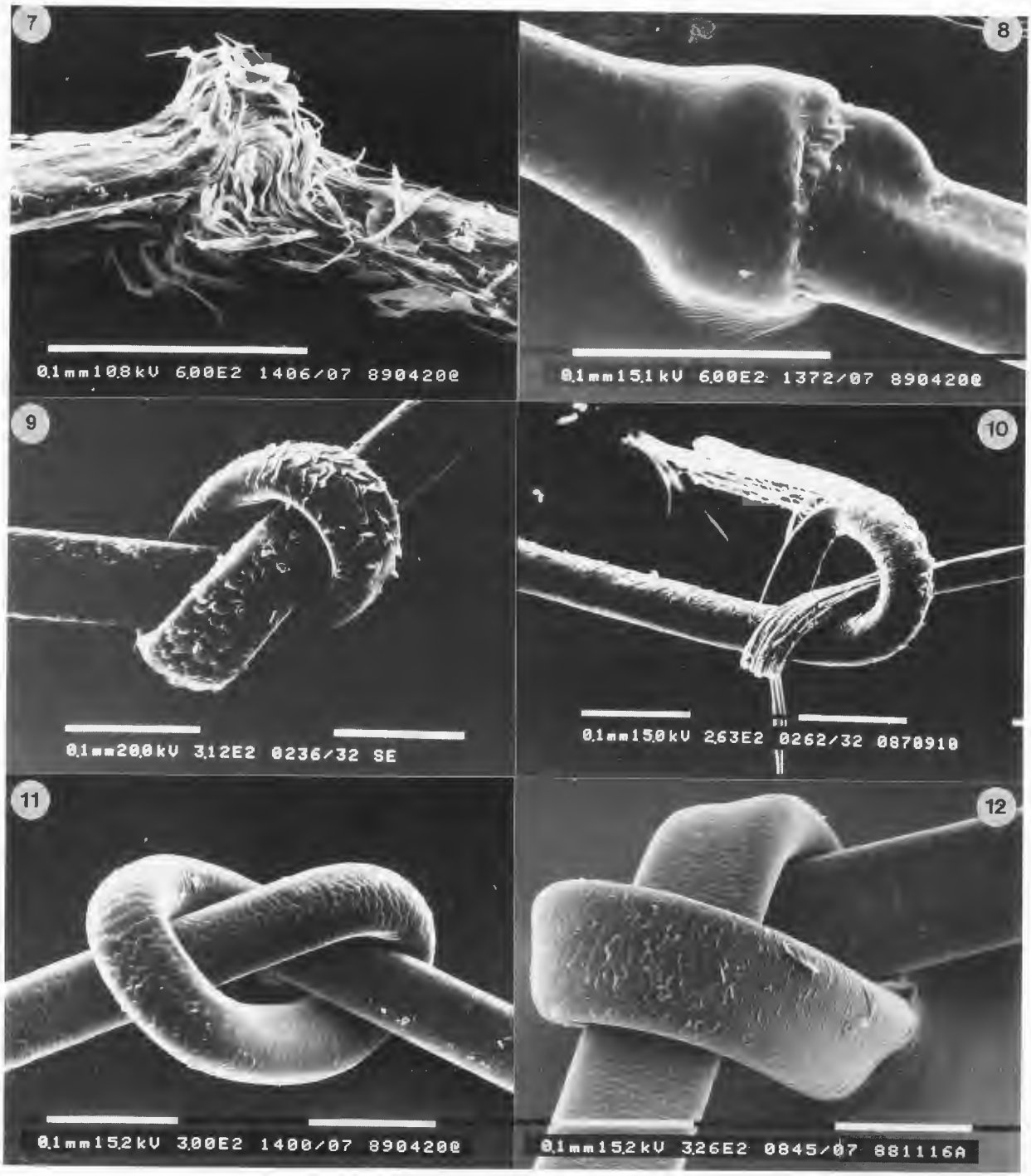

Fig 7. Trichorrhexis nodosa break in hair fiber from a case with abnormally low sulfur content of hair fibers. White bar 100 μm.

Fig 8. Bamboo hair. White bar 100 μm.

Fig 9. Hair fiber with deficient adhesion of cuticle cells. Also notice the slight charging at the free edges of such cuticle cells. White bar 100 μm.

Fig 10. Break at knot on a pathological hair fiber. White bar 100 μm.

Fig 11. Hair fiber with an almost circular cross section reveals curved profiles at the bends in the knot. White bar 100 μm.

Fig 12. Conspicuously flattening at the bends of the knot in a fiber with a medulla. The knot even shows a shallow groove at the bend. White bar 100 μm.

7. **Forslind B, Wiren K, and Malmqvist KG (1987)** Assessment of qualitative and quantitative data from pathological hairs - A critical evaluation of scanning electron microscopy and proton induced X-ray emission analysis. Scanning Microscopy **1**: 159-167.
8. **Forslind B (1988)**. Clinical applications of scanning electron microscopy and energy dispersive X-ray analysis in dermatology - an update. Scanning Microscopy **2**: 959-976
9. **Gerdes RJ, Brown AC (1974)**. Analysis of hair surface structure for product evaluation In: AC Brown (Ed.) The first Human Hair Symposium. Medcom Press, New York, pp 302-316.
10. **Holt LA, Saunders JA (1986)**. A fluorescent microscopy study of the diffusion of some nonionic compounds into wool from solvent mixtures. Text Res Inst J **56**: 415-418.
11. **Marshall RC, Gillespie JM (1982)**. Comparison of samples of human hair by two-dimensional electrophoresis. J Forensic Sci Soc **22**: 377-385.
12. **Orfanos C, Montagna W, and Stuttgen R** (Eds.)**(1981)**. Hair Research. Springer Verlag, Berlin.

Discussion with Reviewers:

D.W.Gregory: Don't you find that double-sided tape is unstable in the beam, leading to movement of the hair during examination? I have found a tiny drop of silver dag or carbon dag to be very satisfactory for adhering hair to a stub.
Author: I agree that the carbon dag mounting technique is excellent in the hands of the erperienced microscopist. However, I advocate the use of tape mounting for the less experienced scientist. We have found that when we work at low acceleration voltages e.g. 5-15 kV, and we ensure that there is good contact between the sputtered coating and the stub metal, we experience no trouble using double-sticking tape as the mounting medium.

D.W. Gregory: Sputtered platinum gives finer granularity coating than gold/platinum alloy. High vaccum evaporation tends to be directional. Do you not find that unidirectional, low-angle evaporation leaves points uncoated which charge in the beam ?
Author: If your problem requires a uni- or bi-directional low-angle coating not seldom you will find that there are areas which charge due to the lack of a complete coating. Again, if it is possible to work at low acceleration voltages charging will be negligible.

W. Wilborn: What does SEM and EDX reveal for hair in Menkes Disease?
Author: The topographic morphology of the hair surface will reveal the **pili torti** changes that are common in this disorder. I have no knowledge of EDX analysis of hair from Menkes syndrome patients. Particle probe analysis (EDX or PIXE) could give very interesting information on the content of **S** along the hair fiber. Especially the Cu/Zn quotient would be interesting to study as Cu has been reported to be abnormal (c.f. several entries under Menkes-Syndrome in CE Orfanos, **Haar und Haarkrankheiten**, Gustav Fischer Verlag, Stuttgart, New York, 1979).

W. Wilborn: Can you provide any new information by SEM and EDX on the corkscrew hairs associated with congenital alopecia due to pili torti?
Author: To my knowledge there is no such information at hand. As is the case when the mechanical properties of a hair fiber are changed it would be of interest to study the effects of forming a knot on the fiber.