A New Staining Method for Visualization of Keratin Filaments in Hair Fibre Cross Sections

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A new method of staining the keratin filament matrix allowing a visualization of the filaments in cross section of hair fibres has been developed. It differs from previously published methods in that the hair fibres are neither fixed with OsO_4 nor treated with sulfur bond breaking agents. High contrast is still obtainable in sections thicker than 60 nm. ***Key words:*** **Electron microscopic staining.**

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The keratin filament size and organization was investigated by transmission electron microscopy already three decades ago. Special techniques involving the reduction of cystin disulfide bonds were developed to visualize the low sulfur filament structures embedded in the random coil proteins of the sulfur rich matrix (1,2). The remarkable physical strength of hair fibres were later shown to be related to the filament organization and the cellular architecture of the cortex and cuticle cells (3,4). Such information provides a background for the understanding of disorders involving changes in keratin formation and cellular adhesion.

We report here a new method for the visualization of keratin fibrils in hair fibre cross section in unfixed specimens avoiding the protein degradation effects of osmium tetroxide (OsO_4) and reductive or oxidative substances aiming at breaking disulfide bonds.

MATERIAL AND METHODS

Hair fibres were plucked from healthy persons in the age range 40–56 years. Specimens for the experiments were taken close to the follicle end of the fibre to ensure least possible influence of environmental factors on the physical/chemical properties of the hair. Wool fibres from a Swedish strain of sheep were selected with corresponding criterions.

Hair snippets were either immersed in 2.5% glutaraldehyde in phosphate buffer at ambient temperature and subsequently post-fixed in 2% OsO_4 for one hour, with appropriate rinses in destilled water. Alternatively the water-swelled specimens were taken directly to dehydration in graded ethanol before embedding in Vestopal. Sections were cut on an LKB Ultrotome fitted with a diamond knife and at a nominal thickness of 60 nm. Sections were stained with aquous solutions of uranyl acetate and lead. Electron microscopy was performed on a Phillips EM 301-G with a 25 μm gold objective aperture at primary magnifications of 10 000, 17 000 and 22 000 ×.

RESULTS

In comparison with specimens fixed in glutaraldehyde and OsO_4 the unfixed specimens yielded far better detail in the keratin fine structure. The best contrast was obtained in relatively thick sections, or section areas thicker than the nominal 60 nm (Fig. 1). The keratin filaments, known from X-ray diffraction studies to have a diameter of approximately 8 nm, were depicted as rounded open spaces in a stained, structureless matrix. The helical arrangement of filaments in the bundles (3) is clearly demonstrated by the "fingerprint whorl patterns" seen in the cross sections. Cellular envelopes ("membranes") are well delineated. There are no signs of filamental structures in the cuticle.

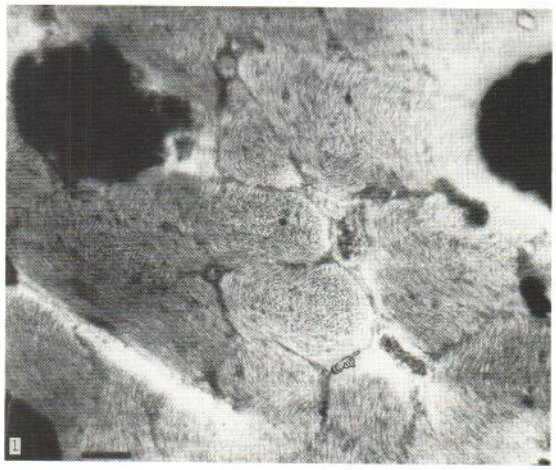

Fig. 1. Cross section of a non-fixed human hair fibre. Section stained with uranyl acetate and lead. Note the "finger print whorl pattern" at ⌀. Bar: 0.1 μm.

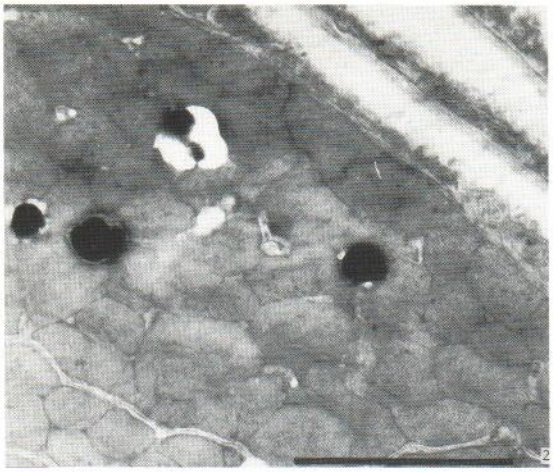

Fig. 2. Cuticle and cortex of unfixed hair specimen. Section stained with uranyl acetate and lead. Bar: 1 μm.

COMMENTS

Our aim has been to develope a method which can be easily performed even by someone not specially devoted to electron microscopy. The method should involve a minimum of health hazards.

The human hair fibre is an almost ideal specimen for analysis in the transmission and the scanning electron microscopes in that it actually needs no special fixation. Previously used methods for the visualization of keratin filaments of the hair cortex have all relied on a pre-treatment with chemicals aiming at disrupting the disulfide bonds of cystin molecules characteristic of hair keratin. The sulfhydryl groups available after such harsh treatment are easily reacted with OsO_4 to provide a very high contrast in the transmission electron microscope. However, the degradation of proteins in situ causing this contrast may provide us with a more artifactual picture of the keratin organization than does our new method. Further, these procedures require special attention to provide successful results. Also, OsO_4 is hazardous to the heath due to its properties as a fixation agent. Since it is volatile special ventilation is an unconditional requirement when working with this fixative.

Our present results show that, in contrast to previous belief, the fine details of hair and wool keratin organization in fibre cross sections may be depicted without a previous chemical treatment aiming at disruption of disulfide bonds. Details such as the so called "finger-print whorl pattern" indicating a helical arrangement of filaments in the bundles are clearly seen in our micrographs. Like previously used preparation methods our technique does not allow visualization of the straight, cuticle filaments. The contrast in hairs fixed in glutaraldehyde and subsequently post-fixed with OsO_4 was remarkably low compared to the unfixed specimens. The logical conclusion is that the cross-linking induced by the glutaraldehyde fixation prevents the uranyl acetate to interact with the proteins to any appreciable extent. Therefore the subsequent staining with lead which mainly is deposited on the uranyl also fails to contribute to the contrast.

At electron microscopy we have found that the best contrast is obtained in sections thicker than 60 nm (600 Å). Due to the difficulty of sectioning hair we recommend that the nominal thickness is set to approximately 60 nm for the best result. If the nominal section thickness is set to 100 nm or more such thick sections tend to burn under the impact of the electron beam. Our experience is that a thicker part of a "thin" section generally will be more stable during electron microscopy than homogeneously thick (>100 nm) sections. Our new preparation method has the advantage of involving few health hazards. The main chemical used, an aquous solution of uranyl acetate, is not volatil and hence no health hazard to any person involved.

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REFERENCES

1. Birbeck MSC, Mercer EH. The electron microscopy of the human hair follicle. J Biophys Biochem Cytol 1957; 3: 202–233.
2. Rogers GE. Electron microscopy of wool. J Ulstrastruct Res 1959; 2: 309–330.
3. Forslind B, Swanbeck G. Keratin formation in the hair follicle. I. An ultrastructural investigation. Exptl Cell Res 1966; 43: 191–209.
4. Forslind B. The growing anagen hair. In: Orfanos CE, Happle R, eds. Hair and Hair Diseases. Berlin, New York: Springer Verlag, 1990: 73–97.