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Individual Characteristics of Chemically Modified Human Hairs Revealed by Scanning Electron Microscopy

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ABSTRACT: Hair that is treated with several different chemical reagents including those that are proteolytic, denaturing, or disulfide bond-reducing agents, undergoes structural alterations both internally and externally as revealed by scanning electron microscopic analysis. Some of these agents produce varying degrees of morphologic alterations in hairs obtained from different individuals. It would seem that this technique can be useful in the discrimination of human hairs from different individuals, since the chemically induced topological changes on the hair shaft apparently exhibit a high degree of intraindividual consistency.

KEYWORDS: criminalistics, microscopy, hair, human identification

Hairs occur frequently as physical evidence in nonviolent as well as violent crimes. It has been estimated that people lose on the average about 100 hairs per day [*1*]. Hairs can fall unnoticed during a crime and remain at the scene as silent witnesses to the event. When recovered, examined, and properly interpreted, they can provide useful evidence [*2*]. Their potential value in this regard is probably much greater than is actually realized in practice, because of the lack of precise and objective methods for hair comparison.

The criminalist is often asked to examine and compare evidential hairs from the scene and known hairs from the suspect and the victim to determine the likelihood of the existence of a common origin between the known and questioned hairs. Such data can be used to suggest a link between the victim or the crime scene and the suspect. Hairs possess a number of morphological features that can be used; however, there is no general agreement as to which one of these is of the most value in discriminating hairs from multiple sources as opposed to a common source. Despite the many microanalytical techniques that have been developed and used to aid in the individualization of hair, most have been found to be inadequate. The microscopical approach in which morphological characteristics are examined has been the method of choice. However, in addition to being time-consuming, this approach yields results that are essentially subjective and of limited value, since one cannot state with anything resembling absolute certainty that a hair sample was derived from a particular in-

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dividual. There is clearly a need to develop improved methods to enable the criminalist to individualize hair evidence or at least approach this goal more closely.

The scanning electron microscope (SEM) can be used to study hair surface topography [*3,4*] in greater detail and with greater depth of field than is possible with the light microscope. This report demonstrates that following treatment of hair samples with mercaptoacetic acid there are alterations in surface morphology that can be visualized and evaluated by using the SEM.

The mechanism underlying these changes is unknown, but because these chemically induced structural alterations are distinctive and appear to depend on the individual source, this technique would seem to have great potential value to the criminalist.

Materials and Methods

A scanning electron microscope (JEOL model JSM 35) was used for the examination of the specimens. All the specimens were treated and examined under similar conditions, using an accelerating voltage of 25 keV and ×500 magnification unless indicated otherwise. Polaroid positive/negative film (PN55) was used for the electron micrographs.

Samples

Anagen stage hairs were collected from 30 males and females who ranged in age from 20 to 60 years old, half of which were obtained from individuals of Asian descent and the remainder from Caucasoids. Three specimens of intact scalp hairs (including root) were obtained in a random fashion from each individual. Using the root as a reference point, a 10-mm length of hair was cut from each specimen with a surgical knife for use in the subsequent examination.

Chemical Treatment

Before the chemical treatment, hair specimens were washed three times (5-min duration for each wash) with 100% acetone to remove possible contaminants such as grease, chemicals, dust particles, hair sprays, and the like. To aid in the selection of a suitable reagent, hair specimens from a single individual were then treated for 12 h with one of the following reagents: 6M hydrochloric acid (HCl), 3M triethyl-aminophosphite in dimethyl formamide, 10% pepsin, 15% sodium carbonate (Na_2CO_3), a saturated solution of urea in 50% Na_2CO_3, *n*-propanol, and 98% mercaptoacetic acid. Some treatments were performed at various pH values, ranging from pH 2 to pH 12. Following chemical treatment, hairs were again washed with water followed by 100% acetone to remove any reagent remaining on the surface. Because it was found that the surface topography of an individual's hair was dramatically altered when mercaptoacetic acid at pH 3.85 was used, all hair samples subsequently analyzed in this report were treated with this reagent at pH 3.85. The hairs were allowed to air dry for approximately 12 h before further processing.

Sample Sectioning

In order to split certain of the hairs longitudinally, a rectangular piece of hard steel, 25 by 76 mm (1 by 3 in.)[2] in dimension, having one finely ground and polished smooth side with an engraved linear groove on its surface, was employed as a cutting jig. Cleaned specimens were embedded in the groove with the help of a cement (Duco brand). The upper protruding portion of the hair was cut away with a razor knife. The remaining embedded hair was removed from the groove by acetone washing and preserved for further treatment.

[2]Original data were given in inch-pound units.

Mounting of Specimens

Standard metallic 10-mm SEM mounting stubs were placed in a stub holder and their surfaces were cleaned by using alcohol swabs. Transparent double stick Scotch® tape (3M Co.) was laid down on these stubs. Excess tape was cut away so that a tape-free surface of the stub would serve as a conductive contact between the specimen and the stub after vacuum coating. Hair samples were mounted on the tape strips and labeled.

Coating

A uniform gold coating of approximately 20 nm in thickness was applied to the mounted specimens by using a Hhummer sputtering unit (Model #HHUMMER 1). Carbon coating of appropriate thickness and gold coating over a primary carbon coating were additional procedures that were used for the surface examination of some hairs.

Results

Preliminary studies were performed on hairs from a single individual to determine what chemical reagent would be most useful in altering surface morphology and therefore result in a distinguishable appearance when viewed with the SEM. It was found that the disulfide reducing agent mercaptoacetic acid at pH 3.85 worked best when allowed to react with hair for a 12-h period before additional washing, drying, and further processing. Figures 1 and 2 illustrate the surface morphology of unmodified human hairs of Asian and Caucasoid origin, respectively. The scale pattern is clearly evident in these micrographs. Figures 3 and 4 are micrographs demonstrating the presence of fine parallel lines superimposed over the scale structure. The hair seen in the micrograph in Fig. 4 comes from a different source than the hair photographed in Fig. 3. These lines were observed in 4 out of 50 samples obtained from different individuals and therefore may be useful in an exclusionary context.

Figure 5*a* and *b* shows carbon/gold coated samples examined under inclined orientation. Examining hairs in this mode is very useful, since hairs from different sources often appear to have different scale patterns.

Figures 6 and 7 show the internal structure revealed by longitudinally sectioning hairs from two locations on the scalp of a single person. This procedure appears to have little or no value in the characterization of hair.

FIG. 1—*Surface morphology of an unmodified Asian hair,* ×*500.*

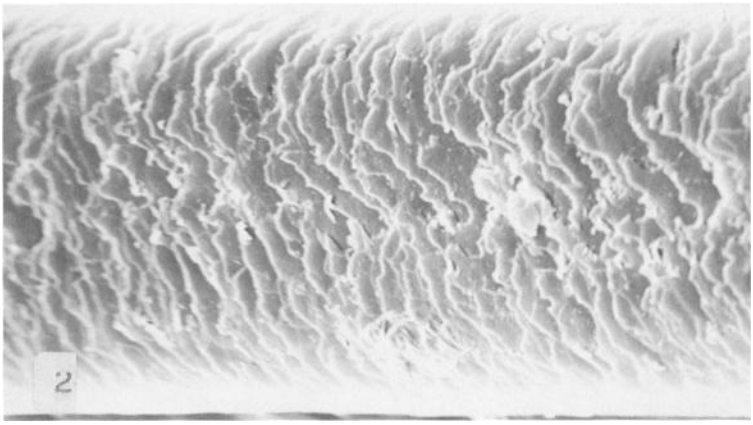

FIG. 2—*Surface morphology of an unmodified Caucasoid hair.* ×*500.*

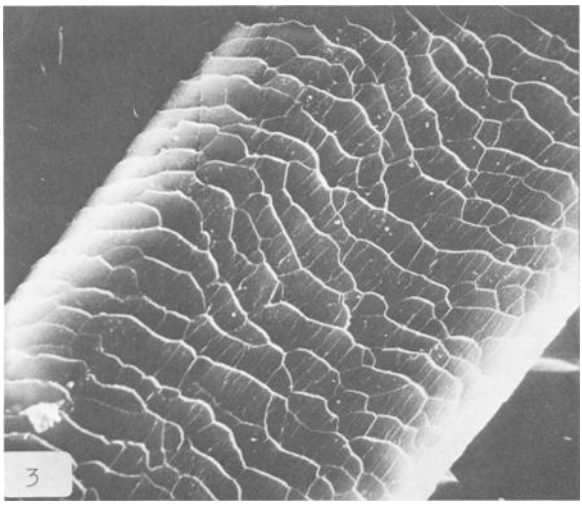

FIG. 3—*Scanning electron micrograph illustrating parallel line scales. Original magnification* ×*500.*

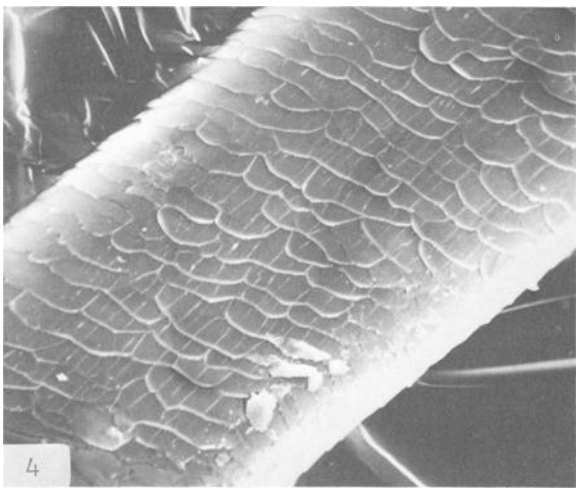

FIG. 4—*Scanning electron micrograph illustrating parallel lines on scales of hair obtained from a different individual than in Fig. 3. Original magnification* ×*500.*

Figures 8 and 9 illustrate the surface morphology of hairs treated with mercaptoacetic acid at pH 3.85 for 12 h. Figure 8*a* and *b* illustrates that the alterations in surface morphology of two chemically modified hairs from the same individual are similar. Figure 9*a*, *b*, and *c* demonstrates that very different surface changes are evident on three hairs taken from another individual and subsequently treated identically to hairs seen in Fig. 8*a* and *b*. It is evident that samples from two different individuals behave differently when treated with this reagent. The observed similarity in hairs from the same individual and the wide variation seen in hairs from different individuals can be exploited in distinguishing hair samples in criminal investigation studies. The altered morphologic characteristics can be classified by the degree of blebbing and cavitation of the surface (Fig. 8*a* and *b* and Fig. 11) or by the appearance of parallel twisted and branched strands (Figs. 9, 10, and 13). Despite limited intraindividual sample variability observed in some micrographs (Fig. 12*a*-*c*), there is a high degree of internal consistency.

In addition to SEM analysis of hair samples, a parallel study using light microscopy was carried out. Untreated hair samples exhibited a wide range of intraindividual differences, particularly with respect to color and pigment granule distribution. The presently proposed technique of SEM analysis of chemically treated hairs appears to be less limited by intraindividual differences than light microscopic analysis and is a potentially useful technique, insofar as hairs from the same source exhibit a much higher degree of internal consistency while samples from different sources showed more obvious surface variations.

Discussion

The traditional approach to hair comparison using light microscopy is obviously useful in cases where two hairs are structurally dissimilar to the degree that an individual can be readily excluded. However, this technique generally provides equivocal results in cases where exclusion is not readily possible. SEM analysis may be a more useful method when used in conjunction with chemical modificiation of the hair surface morphology. Interindividual variability in the structure and chemistry of hairs seems to be accentuated by some chemical treatments to yield topological differences that are easily recognizable in SEM micrographs. Of all the chemical reagents tested, mercaptoacetic acid at pH 3.85 induced the most

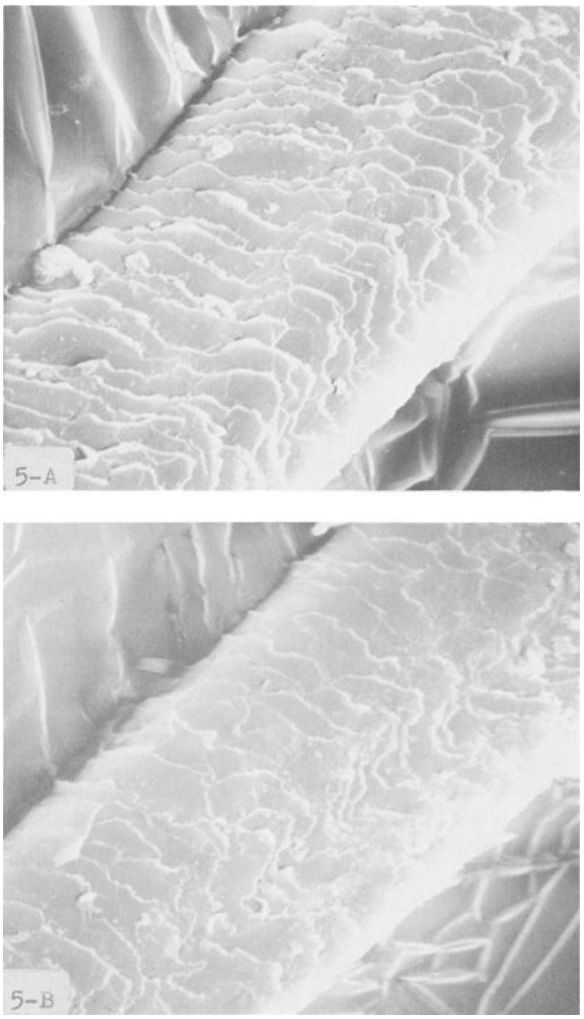

FIG. 5—*Scanning electron micrographs of carbon/gold coated hairs from two individuals. Original magnification ×500.*

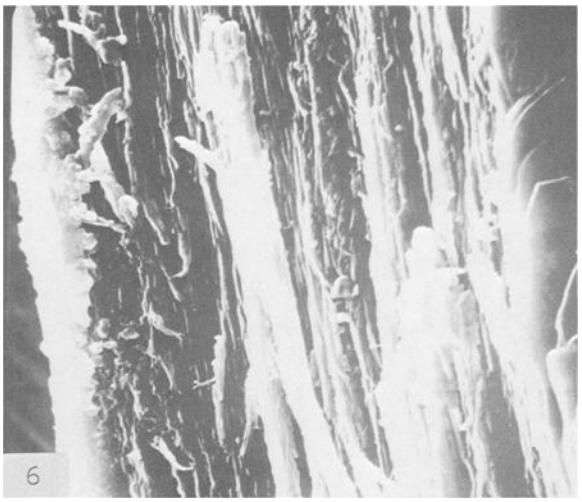

FIG. 6—*Scanning electron micrograph of the internal structure of a longitudinally sectioned hair. Original magnification ×500.*

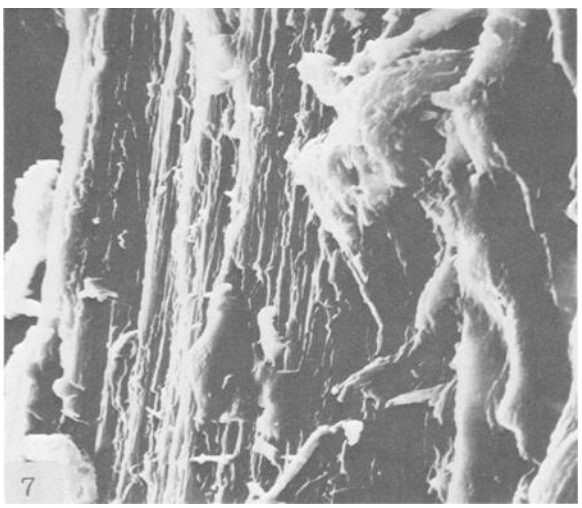

FIG. 7—*Scanning electron micrograph of the internal structure of a longitudinally sectioned hair obtained from a different location on the scalp but the same individual as in Fig. 6.*

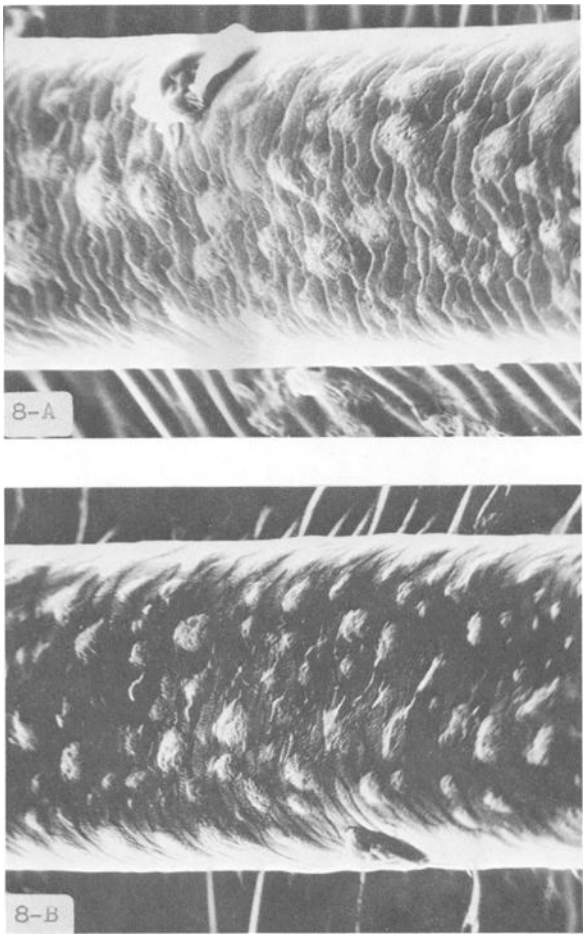

FIG. 8—*Scanning electron micrographs of two hairs from a single individul following a 12-h treatment with mercaptoacetic acid at pH 3.85. Alterations of surface topography are evident. Original magnification* ×*500.*

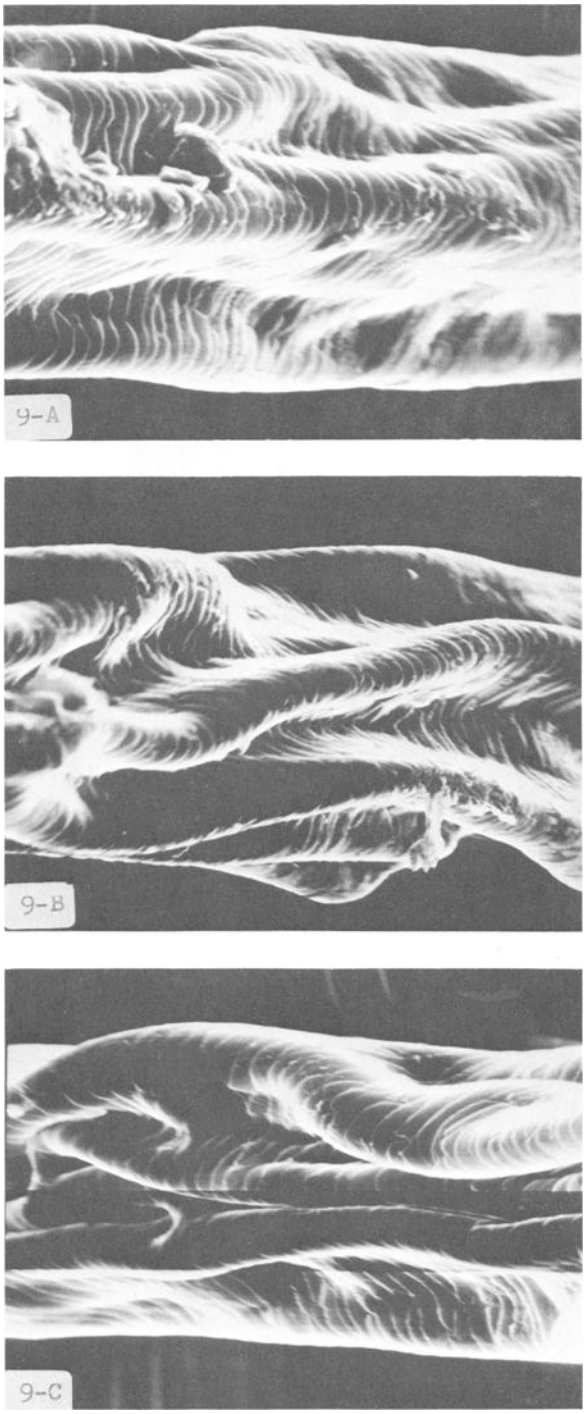

FIG. 9—*Scanning electron micrographs of three hairs from a single individual (not the donor of the hairs in Fig. 8) following 12 h of treatment with mercaptoacetic acid at pH 3.85 illustrating parallel twisted and branched strands. Original magnification ×500.*

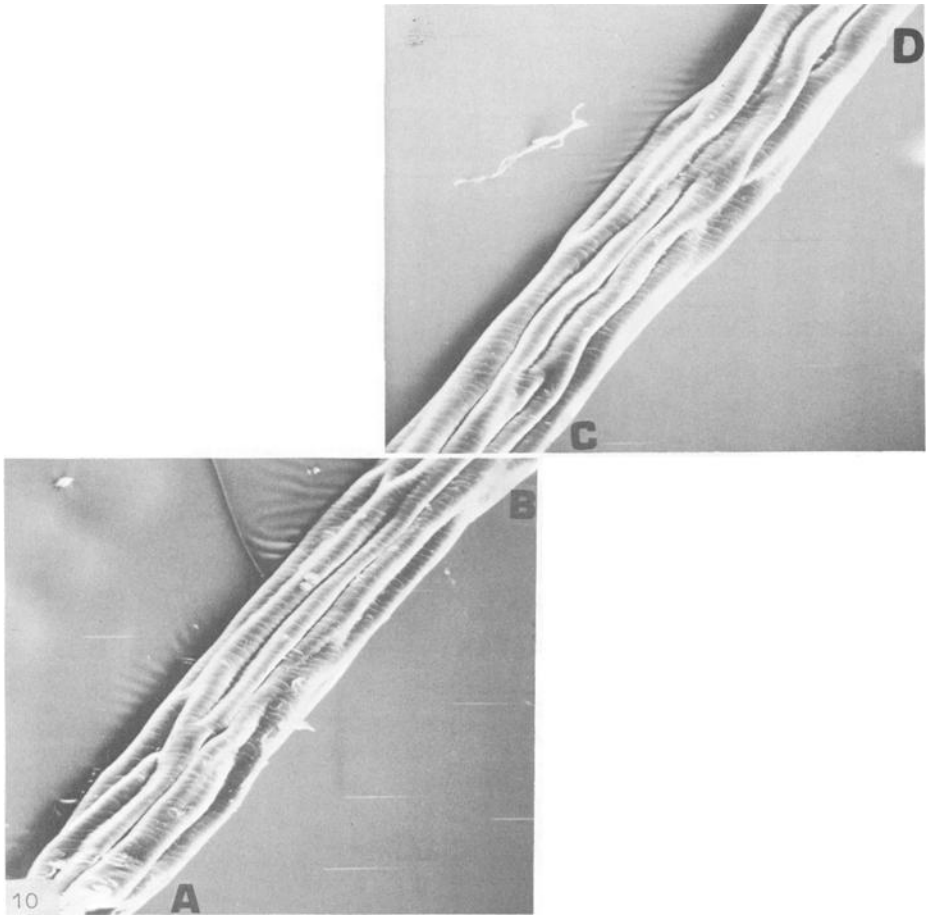

FIG. 10—*Scanning electron micrograph of a single hair following a 12-h treatment with mercaptoacetic acid at pH 3.85. Original magnification* ×*200.*

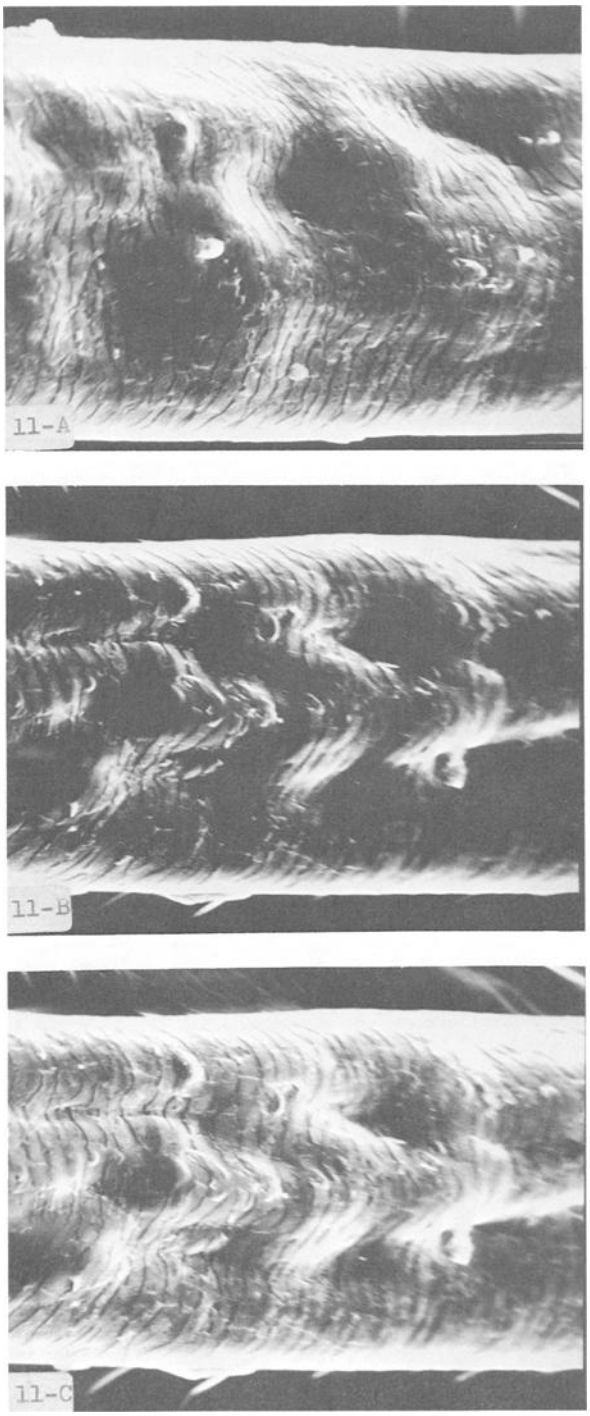

FIG. 11—*Scanning electron micrographs of hair following mercaptoacetic acid treatment, showing the blebbing and recesses of the surface. Original magnification ×600.*

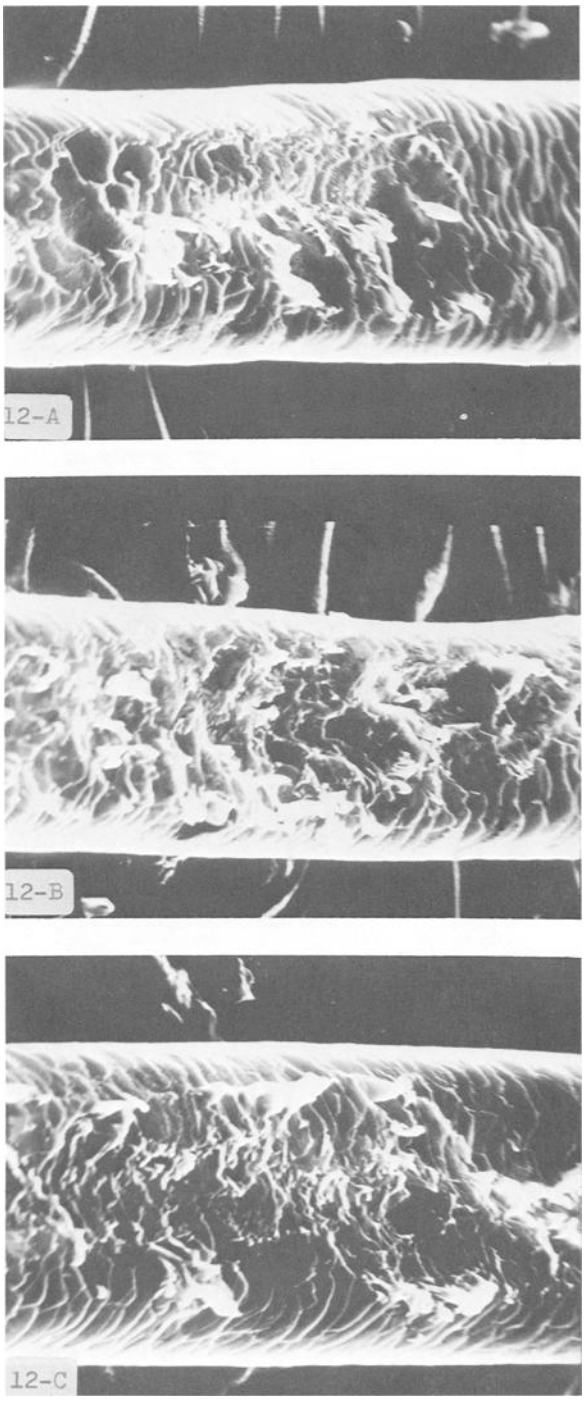

FIG. 12—*Electron micrographs of three hairs obtained from a single individual following treatment with mercaptoacetic acid. Original magnification* ×*500.*

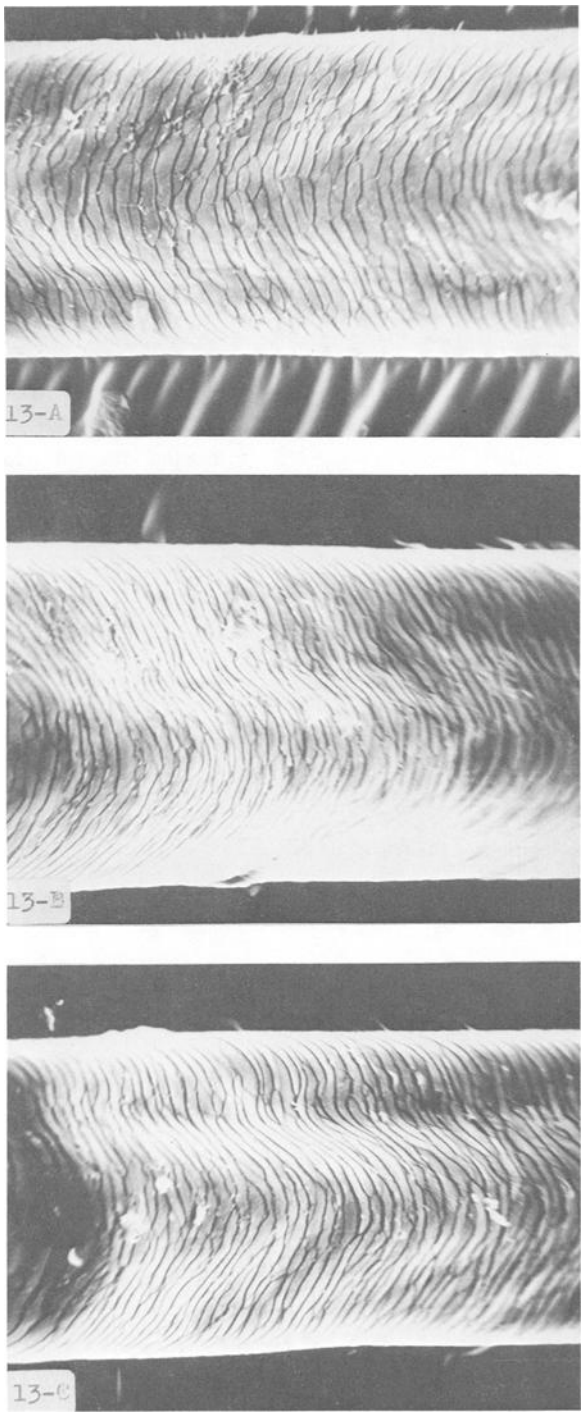

FIG. 13—*Electron micrographs of three hairs obtained from a single individual and treated with mercaptoacetic acid to alter surface topography. Original magnification* ×*500.*

dramatic surface changes in hairs from a single individual. Hair samples from 30 sources were differentiated by electron microscopy by using this disulfide reducing agent (electron micrographs not shown). Light microscopical studies of untreated hairs demonstrated considerable intrasample variability. In some cases this variance was as large as the variance among samples obtained from different individuals. Further, many of the samples from different sources appeared structurally similar when examined by the optical microscope. These observations suggest that the SEM approach could be of considerable value in forensic science casework. It is clear, however, that a much larger number of samples will have to be examined before the system can be fully evaluated. The proposed method of using chemical treatments followed by SEM examination appears to have the ability to eliminate many of the uncertainties in hair comparisons using conventional techniques. The characteristics which the analyst bases his or her opinion can apparently be represented in a single photomicrograph, which is clearly an advantage with respect to court presentation. The method is strictly speaking destructive of the sample; however it is worth noting that the structure remaining following the chemical treatment is stable. No detectable changes were observed in samples that were reexamined three months after the treatment. Thus, the samples would be available for a subsequent examination by a defense expert should it become necessary.

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