TECHNICAL NOTE

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An Improved Method for the Histological Preparation of Single Hairs and Dust Samples—Morphological and Immunological Examination

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ABSTRACT: An improved method of the serological and morphological investigation of human hair is reported. The hair was firmly fixed onto a microscopic slide with cellophane tape and observed microscopically to confirm the cuticula images and the presence of the medulla. A piece of the hair containing the medulla was dissected, embedded in paraffin, and a cross section of this hair was prepared. By treating the sample with immunohistochemistry (biotin-antibiotin ABC technique), the blood type of the hair was confirmed definitively. Dust containing shaved beard can be examined in the same way.

KEYWORDS: forensic science, hair, genetic typing, immunohistochemistry, blood groups, cellophane tape, dust samples

The morphological examination of minute materials, such as dust samples and single hairs, is one of the most important problems in forensic science examination. Recently, Barna et al. and Petraco have derived useful tools for this examination [*1,2*]. We have shown that the cellophane tape method gives some distinct information with respect to not only morphological, but also immunological, investigation of the trace evidence [*3*]. These results will be reported briefly.

Although the absorption elution method, mixed agglutination method, and others for the serological diagnosis of human hair have come to be recognized as some of the fundamental methods used in forensic serology, some difficulties have been encountered in the prosecutional stage, during which defendants have claimed that the recording of the data, expressed by serological scores, could be unreliable [*4,5*]. This charge can especially affect cases in which the specimens have been completely exhausted and their reexamination by other institutions becomes impossible. For this reason, immunohistochemical investigation of hair, which can be performed with minimal damage to specimens and experimental results pre-

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served by permanent histological staining, is expected to be much more effective in the prosecution of criminal cases.

Keeping this point in mind, Miyasaka et al. [6] and Pötsch-Schneider [7] have developed a method that introduces the enzyme-labelled antibody technique (PAP) which succeeds in demonstrating the ABO blood type definitively. Based on the finding that the A and B antigens are mainly located in the medulla tissue, they recommend the staining of a longitudinal section of the hair, as the target tissue of the medulla is often not found in the cross sections of many hairs. Another difficulty encountered in the immunohistological investigation of the hair is in the preparation of histological specimens. They have used celluloid, instead of paraffin, for the preparation of tissue blocks.

Recently, we have established a new system of hair examination by introducing the use of cellophane tape for morphological and serological investigations. Our results will be briefly reported in this communication.

Methods

The hair was placed on a microscopic slide and fixed firmly by covering it with cellophane tape. Microscopic examination of hair enabled the precise analysis of the cuticula images, when the condenser of the microscope was moved to a lower position and its lens narrowed. Figure 1 shows a photograph of the cuticula image under this condition. Thereafter, the condenser was restored to its normal position and the hair specimens, where the medulla tissues were located, were marked, as indicated in Fig. 2. This marked piece was dissected by razor (Fig. 3), embedded directly in the paraffin at the vertical position, and a paraffin section of the hair (a cross-sectioning, in a thickness of 4 to 8 μm) was then prepared. The slice was mounted on a microscopic slide with the aid of "Bond for Wood Working" (acetic vinyl resin emulsion type adhesive, Konishi Co. Ltd. Shudo-cho, Osaka, Japan). The enzyme-labelled antibody technique was prepared according to the ABC method of Hsu et al. [8]. The chart flow of this method is in Table 1.

Results and Discussion

The results are represented in Fig. 4, respectively. The melanin pigment did not disturb the serological reaction and the serological examination of hair containing well-developed medulla tissues, which were not discerned clearly as a core, but distributed diversely. In such

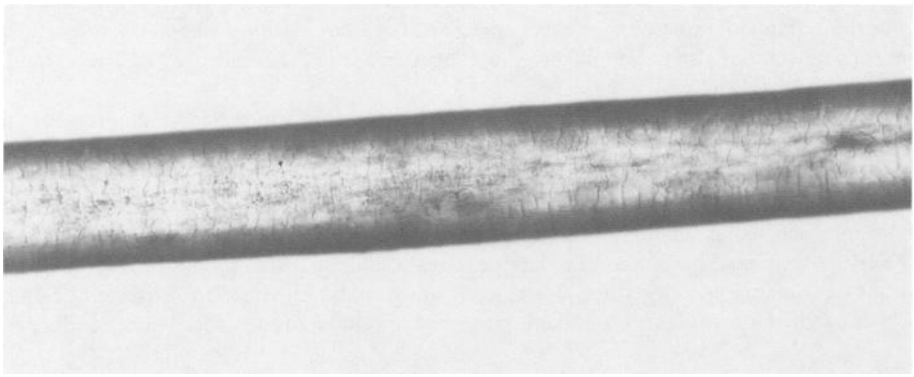


FIG. 1—*Cuticula image. The hair was placed on a microscopic slide and fixed firmly by covering it with cellophane tape. When the condensor of the microscope was moved to a lower position and its lens narrowed a fine thready cuticula image was observed (×100).*

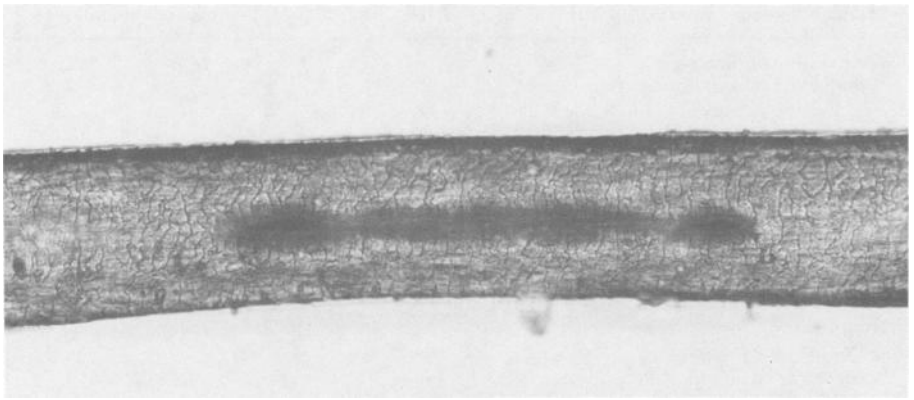


FIG. 2—*Medulla image. The condensor was restored to its normal position. The medulla tissues were located in the hair specimens. It was observed as a black image located discontinuously in the center of the hair (×200).*

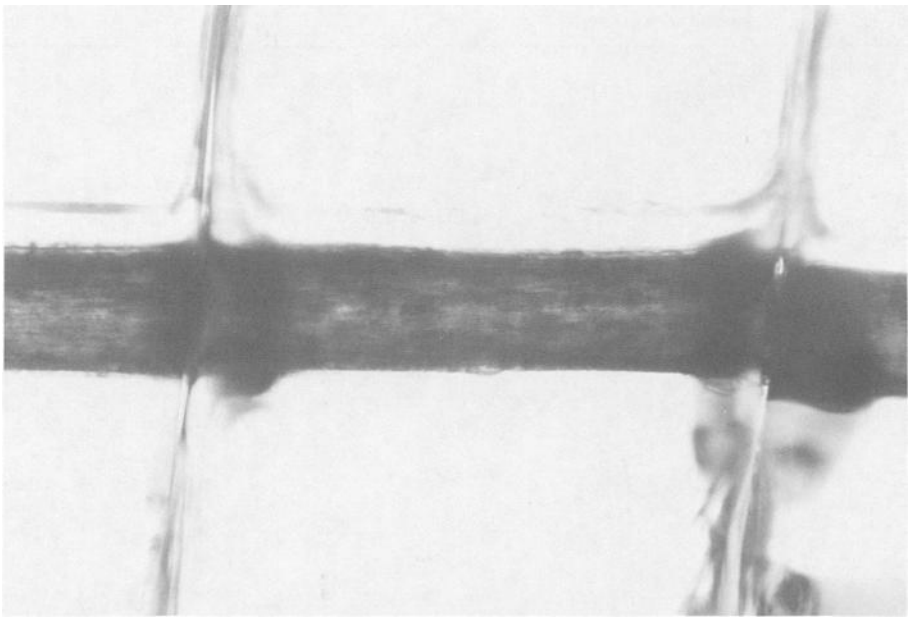


FIG. 3—*The part of the hair containing the medulla held by the cellophane tape was marked and dissected by razor at once (×200).*

cases, the hair containing the rudiments (about 3 mm in thickness) was dissected and a series of 20 sections (0.2 mm in total thickness) was prepared and all specimens reacted anti-A and/or anti-B, respectively. By this method, the diagnosis of the ABO blood type of hair, which showed an apparent lack of medulla, could be performed. The results are represented in Figs. 5 and 6.

The cellophane tape adhesion of a hair sample can also be applied in the serological investigation of shaved beards in either a wet or dry condition. In the cases of dried beards, the

TABLE 1—*Immunohistochemical staining for detection of ABO blood group in human hair.*

1. Preparation of hair samples	
2. Elimination of paraffin by xylol/ethanol	
3. Inactivation of the endogenous peroxidases by H_2O_2-methanol	15 min
4. Rinse in PBS (0.01*M* phosphate buffer containing 0.9% NaCl, pH 7.4)	10 min
5. Tripsin treatment in the concentration of 1%[a]	15 min
6. Rinse in PBS	10 min
7. Pretreatment with 5% normal goat serum or 5% normal rabbit serum (corresponding to primary antiserum)[b]	30 min
8. Primary sensitization[b] Rabbit anti-A (diluted to 1:30) or Goat anti-B (diluted to 1:30)	30 min
9. Rinse in cold PBS	15 min
10. Secondary sensitization[b] Biotinized goat anti-rabbit IgG (diluted to 1:100) or Biotinized rabbit anti-goat IgG (diluted to 1:100)	30 min
11. Rinse in cold PBS	15 min
12. ABC complex[b] (diluted to 1:50)	30 min
13. Rinse in cold PBS	15 min
14. Incubation with 0.005% DAB and 0.002% H_2O_2 in 0.05*M* Tris-HCl buffer (pH 7.2)[b]	
15. Rinse in distilled water	
16. Dehydrate, clear and mount	

[a]Sensitization at 37°C.
[b]Sensitization at room temperature.

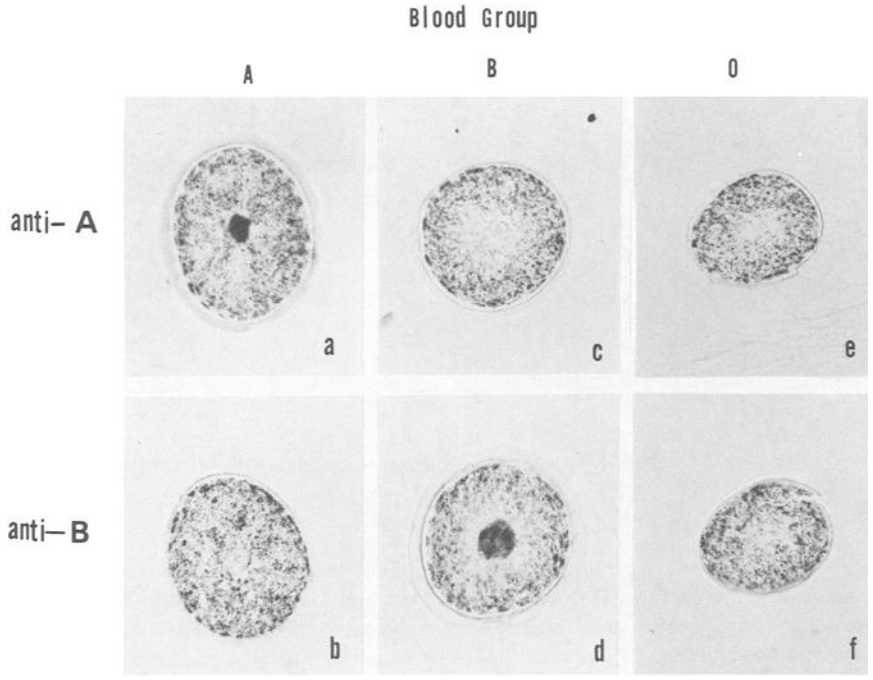
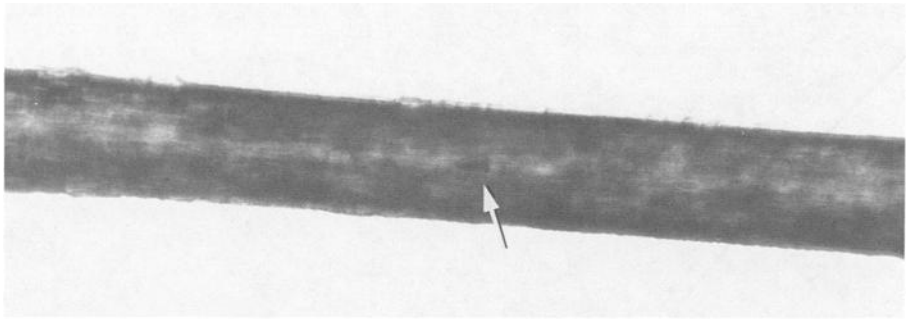
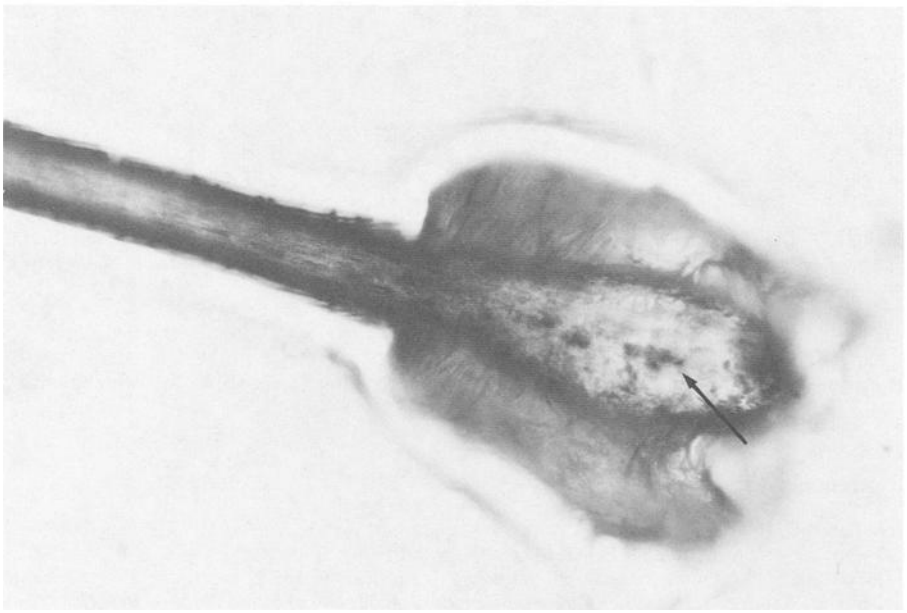


FIG. 4—*Immunohistochemical staining of cross-sectioned black hair. Type-A* (a,b), *type-B* (c,d), *and type-O* (e,f) *hairs immunostained with anti-A* (upper) *and anti-B* (lower) *serum. Positive specific reaction was observed at the medulla. Melanin pigment did not disturb the serological reaction (×200).*





FIGS. 5 and 6—*The case of small medulla. A very small medulla* (arrow) *was found in the center of the hair* (Fig. 5) *and hair root* (Fig. 6). *This was observed as a very small black image. In these samples, ABO blood type could be diagnosed by this method (×100).*

dust was collected on powder and fixed onto the cellophane tape directly. In cases of a wet beard, the shaved beards were washed thoroughly with aqua destilata, dried completely by treating them with ethanol, and fixed onto cellophane tape. The resulting taped piece, containing an abundance of beard, was dissected and treated in the same way. These results are represented in Fig. 7.

Our method has two advantages in comparison to other methods:

(1) excess destruction of the specimens was avoided, so that the remaining hair could be reexamined at any time, and

(2) the hairs, containing trace amounts of medulla, can be examined definitively.

We have already reported that this cellophane tape method in forensic serology and histology is a useful tool in the investigation of trace evidence [*3*]. It is expected that the advance of this method for the preparation of the histology of trace evidence, as we have shown in the

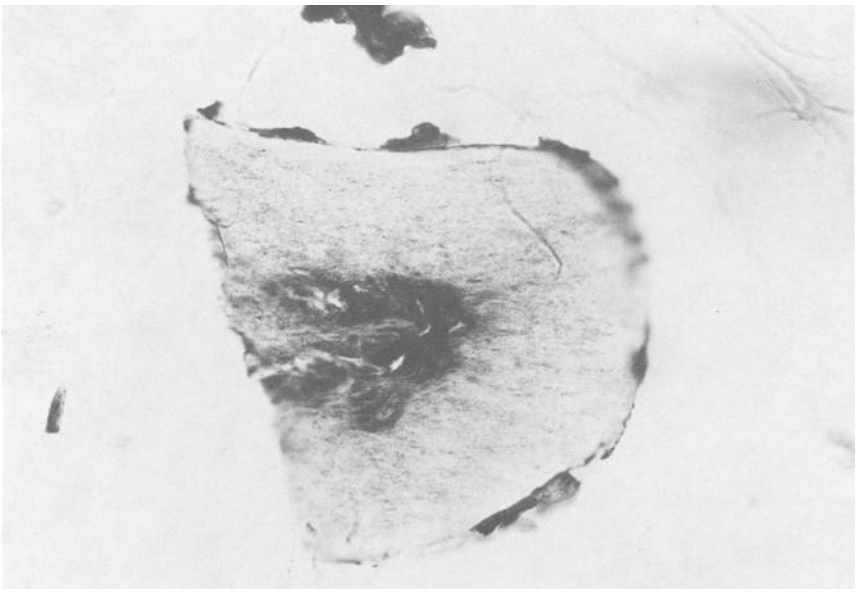


FIG. 7—*The shaved beard sample. The dust was collected on the cellophane tape directly and was dissected and treated in the same way. Positive specific reaction was observed at the medulla after immunostaining (×400).*

investigation of dust, may extend to further research into the field of light and transmission electron microscopy.

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