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Evaluation of the Human Hair Root for DNA Typing Subsequent to Microscopic Comparison*

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ABSTRACT: Telogen human hairs are one of the most common useful evidence findings at crime scenes and/or on homicide victims. Occasionally, the microscopic characterization of the found telogen hair is the only physical evidence association to a victim or suspect. Recently efforts to characterize these hairs by mitochondrial DNA (mtDNA) methods have progressed. The nature of the telogen hair root morphology and ultrastructure has, however, been largely ignored. Examiners have recognized these hairs are unlikely to be typable by nuclear DNA (nuDNA) methods. Most forensic biologists have little knowledge of the complex cellular composition of anagen, catagen, and telogen hair roots or their morphogenesis. This paper reviews ex situ human hair root morphology as it relates to the likelihood of successful nuclear DNA typing. Dermatology texts of hair root morphology always demonstrate their microscopic appearance in the skin. This study investigates the use of fluorescence in situ hybridization (FISH) methods to sex type telogen head hairs, and it further investigates hair root morphology as it relates to the potential nuclear DNA content of evidence hairs.

There is a need for the use of appropriate, consensus terminology for describing hair root morphology. There is also a need for standardized laboratory light microscopic methods in evaluating a hair root for DNA typing. FISH was found to be an unsuitable technique for sex determination of telogen hair clubs. It was determined that anagen/catagen hair roots without translucent sheath material are excellent candidates for nuDNA PCR-based typing and that hairs with telogen club root material only should not be submitted for nuDNA typing attempts.

KEYWORDS: forensic science, hair root morphology, hair growth phases, apoptosis, transmission electron microscopy, fluorescence in situ hybridization, sex determination, hairs, mitochondrial DNA typing, DNA typing, HLA-DQA1, GYPA, HBGG, D758, GC, LDLR

The first essential step of forensic identification and comparison of hairs begins with whole mount light microscopy. Subsequent successful nuclear DNA typing of human head hairs with root tissue (AmpliType PM + DQA1, D1S80, STR and amelogenin) has been widely reported (1–3). Telogen hairs and hair shaft fragments without roots are typable by mtDNA methods (4). One report suggests the use of fluorescence in situ hybridization (FISH) for sex typing of combed human hair root material (5). That study reports successful FISH sex typing for "pulled" and "combed" head hairs. It is unknown what microscopic tissue amounts were present on the "pulled" and "combed" head hairs. No forensic report, however, examines the cellular content of telogen or anagen hairs. The forensic biologist must distinguish possible contaminant material from the many different natural forms an evidence hair root might exhibit. This report examines the complex hair root morphology as it relates to the success of nuclear DNA typing. The FISH method is investigated for its possible use in sex determination of telogen hairs, since these are the type of hairs typically found at crime scenes. Hairs with root tissue (anagen/catagen) are obviously candidates for amelogenin sex typing and the discriminating nuclear DNA markers previously mentioned. These hairs are not considered for the FISH technique.

Mammalian hairs go through three phases of a growth cycle. Human head hairs spend two to eight years in the anagen or active growth phase (Fig. 1). This is followed by about two to four weeks in the catagen or breakdown phase (Fig. 1). The human head hairs then spend about two to four months in the telogen or resting phase until they are naturally or traumatically removed (6) (Fig. 1). In most other mammals, the anagen phase is short and the telogen phase is long (7). Human hair shedding is continuous and not dependent on seasonal change as in lower mammals. It is an error to identify a found telogen hair as necessarily shed because they can also be removed during a traumatic event. Telogen hairs are the most commonly found hairs at crime scenes since the average individual sheds 50–100 hairs during daily activity. The average human scalp contains about 100,000–150,000 hair follicles in various stages of growth or degeneration (6).

Hair root histology is represented by in situ photomicrographs of the follicle and hair in the skin in dermatology texts. The forensic examiner, however, must identify the complex ex situ structures of the found single human hair root. Light microscopic and electron microscopic criteria for identification of telogen and catagen/anagen human hairs ex situ are presented here with the goal of increasing a forensic examiner's prediction of successful nuclear DNA typing.

This study was conducted in three phases to answer two questions. Fluorescence in situ hybridization (FISH) was performed on telogen hairs to determine if this tool could be added to current crime laboratory practices in order to maximize information from the telogen hair (i.e., sex determination). Transmission electron microscopy was used to explore ultrastructural characteristics of anagen, telogen and catagen ex situ hair roots. These findings relate to FISH typing attempts and affect examiner prediction about

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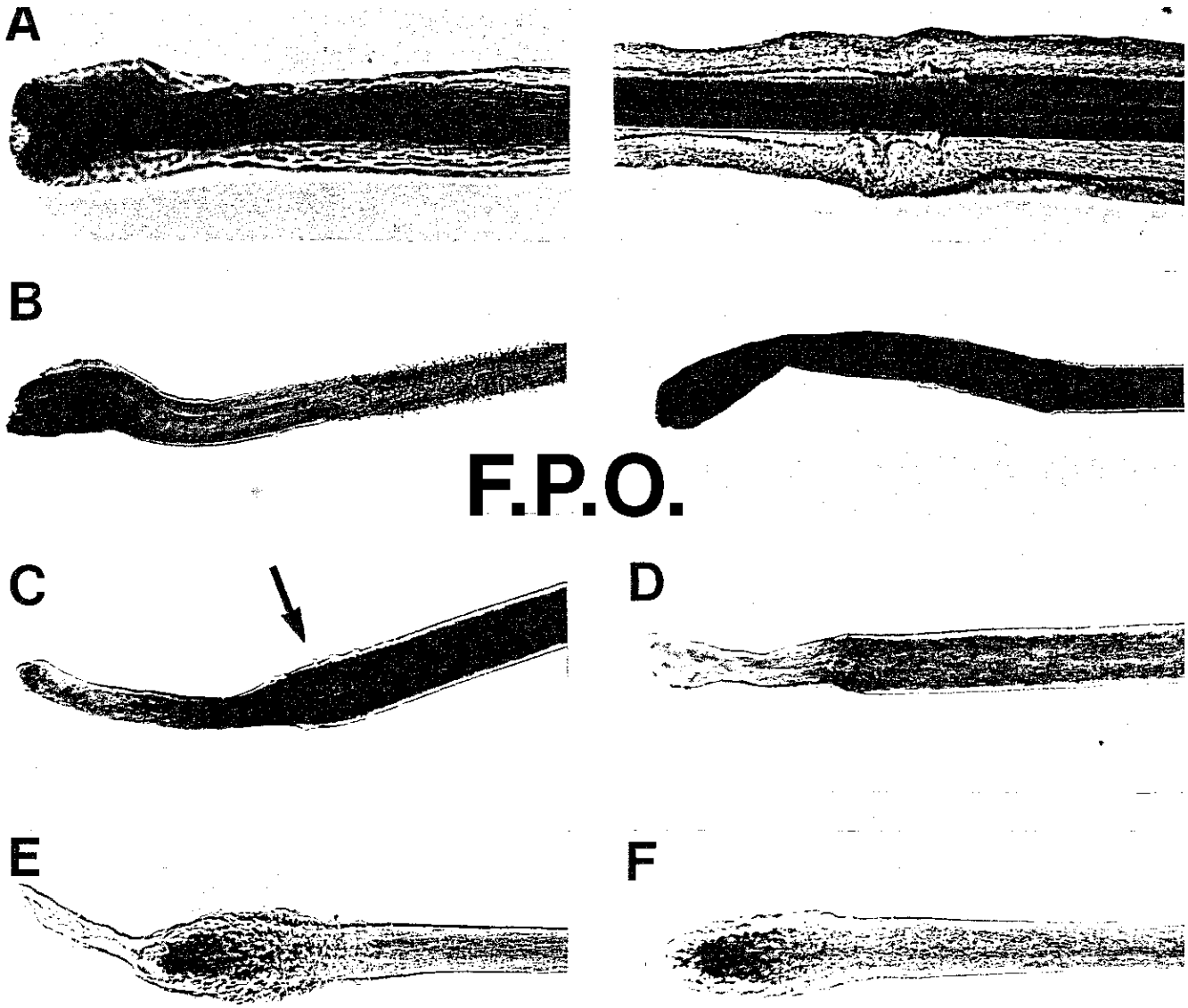

FIG. 1—*Hair root growth and degeneration phases. (A) anagen (active) growth bulb with translucent root sheath (epithelial) tissue. Note flame-shaped, translucent dermal papilla at base of bulb. Sometimes a candidate for RFLP DNA typing if sheath found in excess. (B) Anagen/catagen bulbs (2) with no sheath tissue. Bulb, stem, and cortical shaft. Excellent candidates for PCR DNA typing. (C) Catagen (breakdown) phase root with bulb. Good candidate for PCR DNA typing. Note area of subsequent telogen club formation. (Arrow). (D) late catagen, early telogen phase. (E) Telogen (resting) club with germinal nipple. No usable nuDNA. (F) Telogen club. No usable nuDNA. The process from (A) to (F) results in the total root length being reduced by about two-thirds due to cellular loss (apoptosis), fibrosis, and cornification. Whole mount (Permount) light microscopy. (×400)*

which type hair root ends would yield nuclear DNA PCR product. Finally, PCR amplifications were performed on certain root end morphologies to further investigate nuclear DNA typing success predictability, using a modified hair extraction protocol which involved hair shaft grinding. These PCR findings were compared to a forensic casework retrospective study examining probative evidence hairs that were PCR typed by conventional extraction methods.

Materials and Methods

Fluorescence In Situ Hybridization Sex Determination of Telogen Hairs

The CEP X (α satellite) SpectrumOrange DNA probe hybridizes to the centromere of human chromosome X (bands p11.1-q11, locus DXZ1) and the CEP Y (satellite III) SpectrumGreen DNA probe hybridizes to the satellite III sequence of human chromosome Y (band Yq12, locus DYX1). Both hybridized probes produce fluorescent signals in interphase nuclei and on metaphase chromosomes. Normal male and female control cells are recommended to be placed on the same slide as the specimen when using the CEP X/Y (sat III) assay.

Samples

Telogen head hairs were gently removed from the scalps of a total of fifteen male and female individuals and mounted to glass microscope slides with Permount resin for up to six weeks (whole mount preparations). These hairs were microscopically screened

for those with telogen root clubs and no adherent translucent germ cell tissue for testing. Hairs possessing visible tissue are candidates for amelogenin DNA PCR sex typing and were not considered for this phase of the study. The hairs were removed by scoring and breaking the coverslip, dissolving with xylene and washing with dH_2O. Material was teased from the root club with a sterile scalpel blade in a drop of 60% acetic acid. The root club material was heat fixed to the microscope slide.

Hybridization

The fixed telogen root club material was prepared for FISH using the Vysis Spectrum CEP X (α satellite), Y (satellite III) cocktail probes according to the manufacturers instructions. Briefly, the microscope slides with the telogen root club material were denatured at 75°C for 5 min in 70% formamide/2X SSC. The slides were dehydrated for 1 min each in 70%, 85% and 100% ethanol and were maintained at a temperature of 45–50°C. The Vysis CEP probe mixture of 7 μL hybridization buffer, 1 μL X,Y cocktail probe and 2 μL of dH_2O was maintained at 45–50°C after denaturation at 75°C for 5 min. The dry telogen root club material was treated with 5 μL of probe mix, then coverslipped and incubated for 30–60 min at 42°C in a non-humidified chamber. The coverslip was removed and the slide was placed in a coplin jar containing 0.4X SSC for 2 min at 75°C for post hybridization wash. The glass slide was then washed in 2X SSC/0.1% NP-40 at room temperature for 5–60 s. The microscope slide was dried in darkness and coverslipped after the application of 10 μL of DAPI II counterstain to the telogen club material.

Fluorescence Microscopy

The slides were examined for non-overlapping cells (an important ancillary step to avoid false results) on a Zeiss fluorescence microscope with a triple bandpass filter system for the presence of X (orange) and Y (green) chromosome signals. The microscopist did not know the identity of the hair donor(s). Photomicrographs were taken when possible due to the low emission intensity of some fluorescent signals.

Transmission Electron Microscopy (TEM)

Several telogen head hairs (without germ cell tissue at the root) and several anagen head hairs (without shaft sheath tissue) were immediately placed in glutaraldehyde after removal from the scalp. Routine TEM fixation, embedding, ultramicrotomy, and staining techniques were used. This included osmium tetroxide post-fixation, ethanol dehydrations, propylene oxide infiltration, and embedding with epoxy resins (Araldite, Epon). After ultramicrotomy, the sections were stained with uranyl acetate and lead citrate on formvar-coated grids.

The approximate 80–90 nm thick sections were examined on a Jeol Jem 1200EX II transmission electron microscope operated at 120 kV. Electron micrographs of one telogen head hair club, one anagen head hair bulb with adjacent stem and cortex, and one late anagen/early catagen head hair bulb with adjacent stem and cortex were obtained.

AmpliType PM + DQA1 PCR Amplification and Typing

Samples

Twelve Institute of Forensic Sciences employees contributed one telogen head hair with club root, or one telogen head hair with germ tissue, and one anagen/catagen head hair. These employees had known AmpliType PM + DQA1 typings from blood samples on file at the Institute. These 24 hairs were mounted on glass microscope slides in Permount for up to six weeks. After microscopic screening (×100) and photomicroscopy, a 2-cm section of the hair including the root, was removed by scoring and breaking the coverslip. This area of breakage was dissolved with xylene to facilitate removal of the hair. A sterile scalpel blade was used to remove the proximal portion of the hair including the root.

Modified Hair DNA Extraction

Each 2-cm piece of hair with attached root was placed in a 1.5-mL microcentrifuge tube with the addition of 500 μL of 2% SDS. Hairs were sonicated for 10 min and then washed with dH_2O. Each hair section was placed in a 0.2 mL Kontes glass grinder (Fisher # K885470-0000) which contained 150 μL TE^{-4}. Grinding was performed so that no hair material was visible by stereomicroscopy. The homogenate, approximately 150 μL, was added to 50 μL of 20% (w/v) Chelex®100 (Perkin-Elmer P/N N808-0087). This material was vortexed for 5–10 s and microfuged for about 10 s. The tubes were placed in a dry bath incubator at 100°C for 8 min. The extracted material was vortexed for 5–10 s and microfuged for 2–3 min at high speed.

The careful cleaning of glass grinders has been described previously (4). The glass grinders were cleaned between uses to prevent cross-contamination. Each grinder was used for about 4–5 hairs from different individuals and then discarded.

Amplification and Typing of the Extracted DNA Using the AmpliType PM + DQA1 Kit

The manufacturer's protocol for the AmpliType PM + DQA1 Kit was followed for amplification using 20 μL of extracted hair DNA. The 102-μL total volume mixture (40-μL PM reaction mix, 40-μL primer mixture, 2-μL BSA (8 mg/mL) and 20 μL DNA) was amplified in a Perkin-Elmer GeneAmp PCR System 2400 Thermal Cycler. Five microliters of 200 mM disodium EDTA was added to each tube prior to strip hybridization. Twenty microliters of denatured amplified DNA product was used for the reverse dot-blot, allele-specific oligonucleotide (ASO) strip hybridization. Again, the manufacturer's protocol was used for hybridization and strip development. Strip development was accomplished in a Bellco Glass, Inc., dual pan hot shaker (Cat # 7746-32110).

Results and Discussion

Fluorescence In Situ Hybridization Sex Determination of Telogen Hairs

Results

Out of 15 telogen head hairs from 15 individuals, no telogen head hairs were correctly typed for sex identification by the FISH method. Most cells and cell remnants exhibited no signals, while a few cells exhibited more than two. Some male hair root materials exhibited female signals (two or more orange X chromosome signals fluorescing), and some female hair root materials exhibited male signals (one orange X chromosome signal and one green Y chromosome signal fluorescing). One male and one female fresh telogen hair root club, not exposed to Permount, were also tested and yielded the same false hybridization results.

Fluorescence In Situ Hybridization Sex Determination of Telogen Hairs

Discussion

These findings strongly indicate nuclear DNA degradation. The telogen hair root club results from the shrinkage, detachment, and descent of the viable cellular bulb material beneath it (dermal papilla, matrix cells, stem and fibrous outer root sheath) (7). The late telogen club is encased in trichilemmal keratin and is completely surrounded by the shrunken outer root sheath while in the skin (8). The programmed cell death (apoptosis) of elements below and around the telogen club explains the non-specific probe hybridization described above. Apoptosis results in double strand cleavage of nuclear DNA at linker regions; while in necrosis there is random DNA degradation with digestion of histones (9). Cell shrinkage and nuclear condensation is one of the electron microscopic characteristics of apoptosis (10). Degraded nuclear DNA is not a suitable target for FISH probes since false, absent, or multiple signals may occur (10). Telogen hairs with visible translucent germ (epithelial) tissue surrounding the club were not tested because these hairs would be better candidates for amelogenin PCR amplification for sex determination and, other nuclear DNA markers. The telogen root club has keratinized rootlets which anchor into surrounding germ cells and these translucent cells may accompany the telogen club root upon pulling during early telogen/late catagen (11). This translucent cornifying structure, seen in ex situ hairs, is also referred to as the telogen club nipple (8) (Fig. 1E).

Intact interphase nuclei or metaphase chromosome preparations are a requirement for successful FISH results (10). Intact interphase nuclei do not exist in the telogen root club because it consists of trichilemmal keratin (see Transmission Electron Microscopy Results). This is a soft keratin material as opposed to the downwardly pointed hard keratin of the hair shaft cortex above it. It is worthy to note that anagen hair roots digested with Proteinase-K (20 mg/mL, 4 h at 56°C) and mounted in Permount exhibit the same light microscopic characteristics as the "dead man's" root end (downwardly pointed shaft remnant with dark banding) (Fig. 2). This suggests that the "dead man's" root end morphology is possibly caused by the digestion of the soft bulb by autolytic proteinases released by the surrounding necrotic cells in the decomposing skin. Some hair examiners use the terms "putrid root" and "postmortem root banding" to describe this hair morphology.

While a previous report suggests that FISH might be useful for gender determination of hairs (5), this study shows that FISH is unsuitable for sex typing of found telogen hair clubs. FISH probes have inherent problems even when used with fresh viable cells (10). Loss of target DNA, poor penetration of probe, and incomplete or non-specific hybridization are problems associated with apoptotic, necrotic, and keratinizing cells (10). FISH requires examination of a large number of cells, the use of control cells on the same microscope slide as the evidence slide (due to critical temperature requirements) and sophisticated statistical analysis (10).

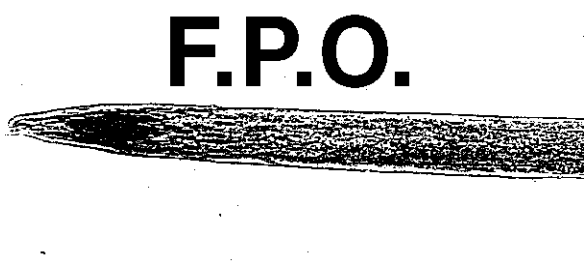

FIG. 2—*Anagen root bulb (Fig. 1-B) after digestion with Proteinase K. Only hard keratin of cortex remains. Bulb and stem tissue has been digested. Black banding is an area of air vacuole formation. Same microscopic appearance as hairs from a decomposing human scalp. Light microscopy, Permount embedding. (×400)*

TEM of Telogen, Catagen, and Anagen Head Hairs

Results

No intact nuclei, nuclear remnants, mitochondria, melanosomes, or keratin filaments were seen in the featureless trichilemmal soft keratin material of the telogen root club (Fig. 3B). Numerous intact nuclei were seen in the anagen and early catagen root bulbs (Fig. 4B). The hard keratin filament density increases as one goes from proximal to distal regions in the anagen stem and cortical shaft (Fig. 5). Nuclei were observed to become increasingly elongated as one goes from proximal to distal regions in the anagen/catagen stem and cortical shaft. (The term "stem" is used here to designate the ex situ slender, pigmented, usually longest section of the terminal hair root that stretches from the summit of a bulb to the base of the hair cortex.) Areas of autophagic vacuole formation were visible in the upper regions of the late catagen bulb and stem.

TEM of Telogen, Catagen, and Anagen Head Hairs

Discussion

Empty vacuoles seen in the telogen club begin to form during the late catagen phase as the surrounding germ cells form a capsule sac around the club. These vacuoles are formed during cornification, a dehydrating process. A fully cornified hair contains less than 10% water (7). These empty vacuoles appear black with whole mount light microscopy (as do air-filled medullary structures and cortical fusi) and should not be incorrectly identified as large pigment accumulations within the translucent club material.

Figure 1A, left, illustrates the viable anagen bulb which contains numerous matrix cells and melanocytes. The egg-shaped translucent dermal papilla protrudes into the base of the bulb. The hair bulb is a structure rich in nuclear and mitochondrial DNA. The rate of turnover of the matrix cells is greater than that of any normal tissue, with the possible exception of bone marrow (7). Scalp hairs grow approximately 0.35 mm per day (7). Figure 5 demonstrates the formation of hard keratin filaments as one moves from proximal to distal regions in the anagen hair shaft. Compare to the featureless soft keratin material of Fig. 3B.

The onset of the catagen phase is not visible with light microscopy ex situ since it is diagnosed by the cessation of mitosis in the bulb matrix cells, the collapse of the outer root sheath epithelium (apoptosis) and the presence of pigment granules in the dermal papilla. (The outer root sheath actually represents a downward extension of the epidermis (8).) It is possible to see catagen hairs in skin that have fully developed club structures (telogen) but exhibit evidence of ongoing catagen activity (12). The whole mount light microscopic distinction between late anagen and early catagen, and late catagen and early telogen, is not always clear. It is of interest to note the future site of telogen club formation in the catagen hair root above the bulb and stem (elongation zone) (Fig. 1C). During the growth cycle from anagen to telogen, the hair follicle shrinks to about one-third of its former length and the telogen club is formed at the level of attachment of the arrector pili muscle (8). These authors assign anagen, catagen, or telogen

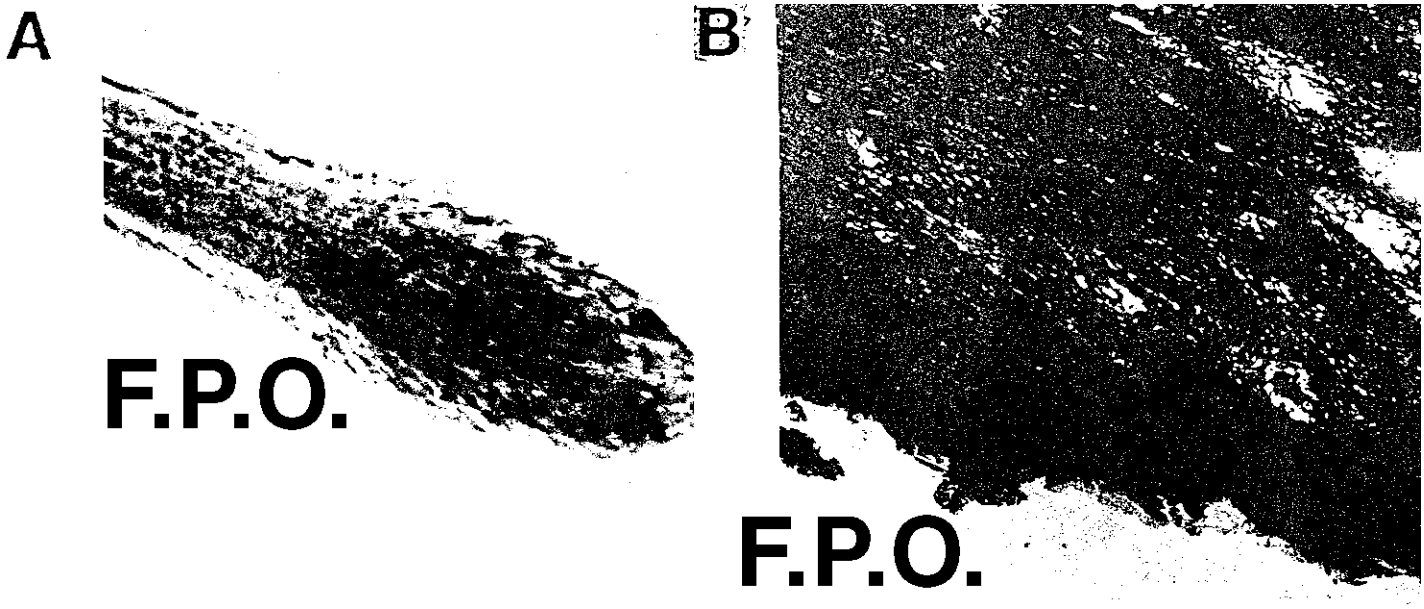

FIG. 3—*(A) Light microscopy of telogen club. Note numerous dark air vacuoles. (B) Transmission electron microscopy of telogen club. The club is composed of trichilemmal keratin. Note numerous air vacuoles and absence of cellular elements. The entire club consists of the homogenous amorphous material. (×1200)*

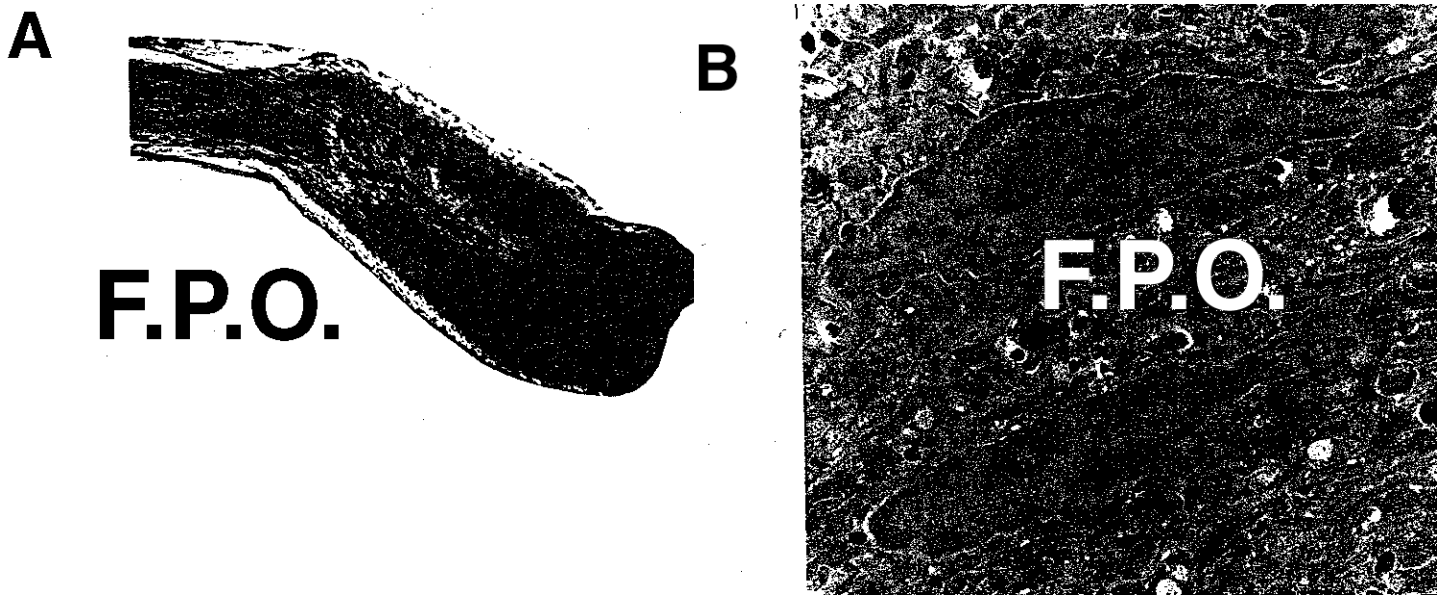

FIG. 4—*(A) Light microscopy of anagen bulb. (B) Transmission electron microscopy of anagen bulb matrix surrounding the dermal papilla. These cells give rise to the cortical cells. Note tightly packed, intact nuclei. The melanosomes (dark inclusions) are produced by melanocytes and are transferred to young cortical cells. (×18000)*

growth status to ex situ whole mount hairs with regard to the dominant root histologic structures present (see Fig. 6).

It should be remembered that the catagen (breakdown) phase of the hair cycle lasts only about two to four weeks and that in the non-balding scalp, approximately 80–90% of the hairs are in the anagen (active growth) phase and about 10% of the hairs are in the resting or telogen phase (6). Naturally shed telogen hairs accumulate over time in the victim or suspect's environment and anagen hairs are more likely to be removed by trauma. As previously noted, telogen hairs can also be lost due to trauma along with anagen/catagen hairs.

AmpliType PM + DQA1 Typing of Head Hair Roots

Results

The AmpliType® Polymarker (LDLR, GYPA, HBGG, D7S8, GC) and HLA (DQA1) amplification typing kit detected no PCR product for head hairs with telogen clubs and head hairs with telogen club and attached germinal nipples (Fig.(s) 1E, F). Telogen head hairs with ample translucent germ/epithelial tissue surrounding the keratin club produced detectable PCR product in 2 of the 3 head hairs tested.

Anagen/catagen head hairs with only a bulb produced detectable

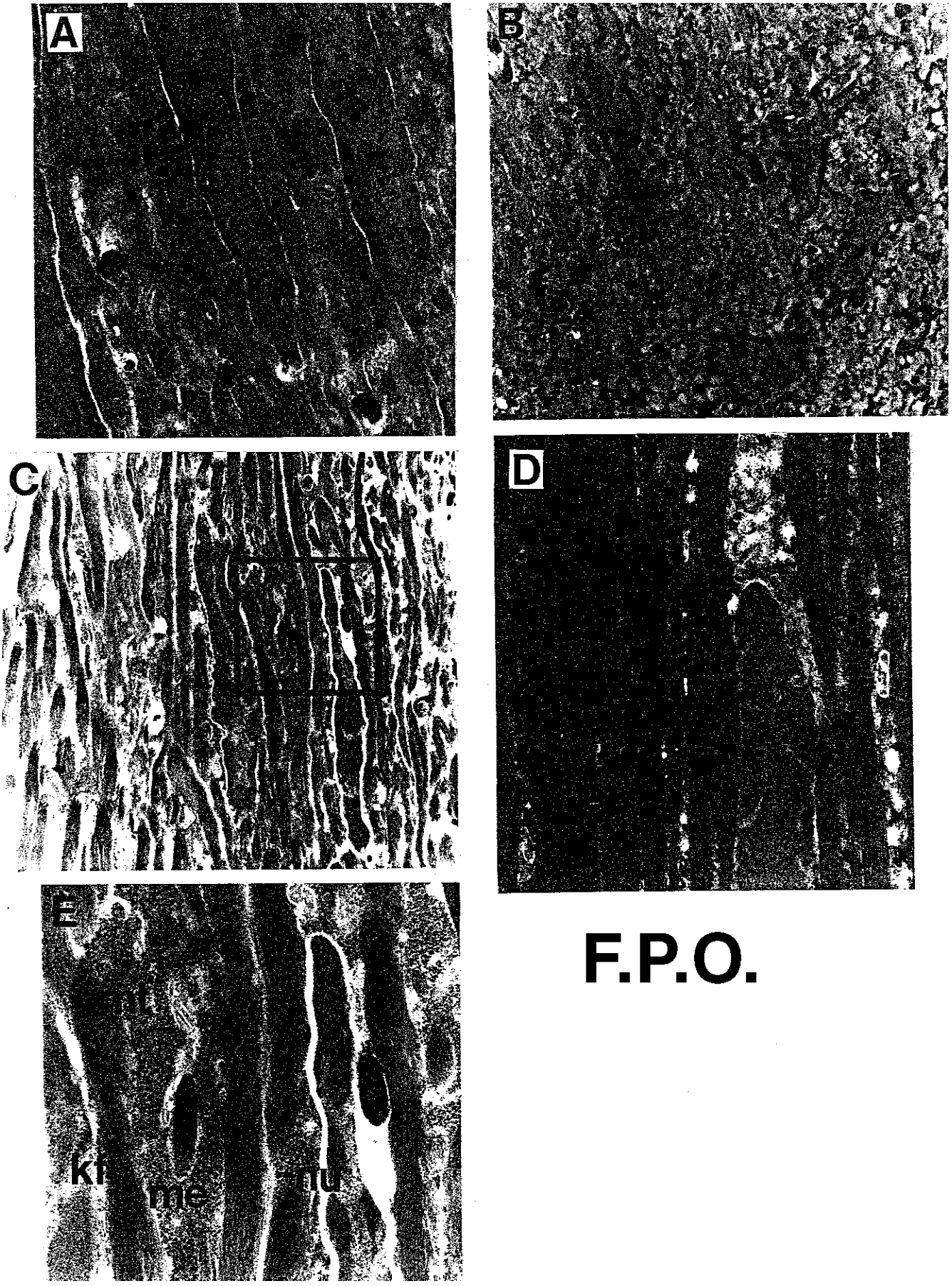

FIG. 5—*(A) Matrix cells of anagen bulb. (B) Higher level of cortical cells transformed from matrix cells. Tonofilaments are being produced. Prekeratinous zone (X6000). (C) Tonofilaments becoming more dense at higher level of shaft than (B) keratogenous zone. (D) Dense keratin filaments of hair cortex with one elongated nucleus visible. (E) Higher magnification of (C). Mitochondrion, mt, keratin filaments, kf, elipsoid melanosome, me, and nucleus, nu.*

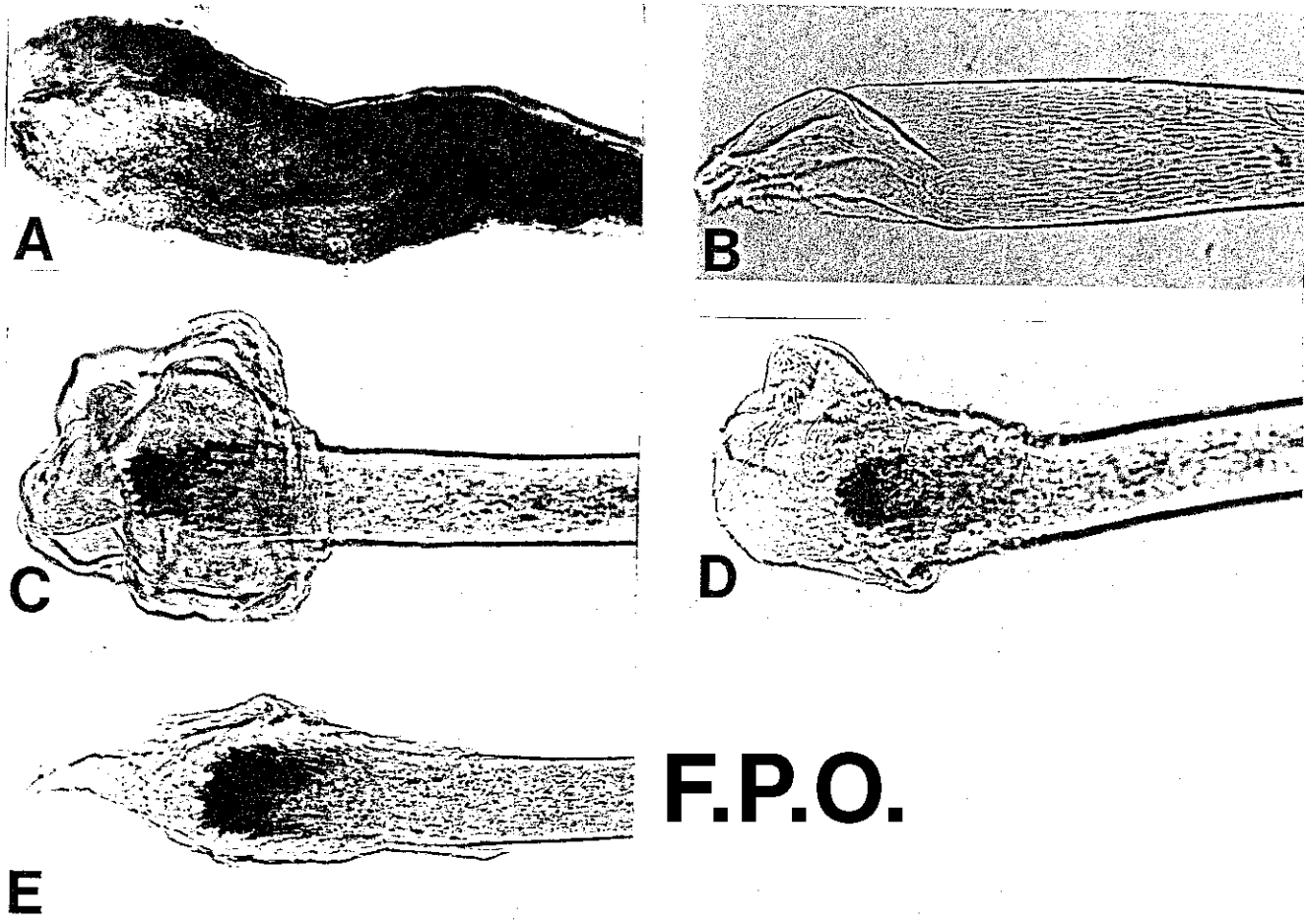

FIG. 6—*Identification of pubic hair root structures. (A,B) Anagen/catagen bulb without sheath tissue. Excellent candidate for PCR DNA typing. (C,D) Telogen club with ample translucent germinal tissue (follicular tag). Good candidate for DNA PCR typing. (E) Telogen club with germinal nipple. Poor candidate for DNA PCR typing. The majority of hairs found in sexual assault cases are like (C,D) because pubic hairs, unlike head hairs, spend more time in telogen phase than anagen phase. Light microscopy, Permount embedding. (X400)*

PCR product in 6 of 7 head hairs tested (Fig. 1B). All head hairs with root sheath tissue and bulb produced detectable PCR product (Fig. 1A). Table 1 summarizes the results from this study. Table 2 summarizes a retrospective study of hair nuclear DNA PCR results which involved probative evidence hairs in Dallas County Institute of Forensic Sciences cases. The retrospective study hairs were amplified over an eight year period, by several different laboratories, using different extraction methods. No labs in the

TABLE 1—*Hair PCR DNA results from this study in which the grinding method of extraction (Chelex 100) was used.*

Type of Hair Root	Number Tested	Number with Detectable AmpliType PM + DQA1 Product
Telogen with club only	6	0
Telogen club with translucent germinal nipple	6	0
Telogen club with ample translucent germinal tissue (follicular tag)	3	2
Anagen/Catagen bulb only (no translucent tissue present)	6	5
Anagen/Catagen with bulb and translucent sheath tissue	3	3
Total	24	10

TABLE 2—*Hair PCR DNA results from Dallas County Institute of Forensic Sciences casework retrospective study. Extractions were done by three different laboratories over an eight year period; but, none utilized the grinding method.*

Type of Hair Root	Number Tested	Number with Detectable PCR Product (Various Genetic Loci)
Telogen with club only	6	0
Telogen club with translucent germinal nipple	3	0
Telogen club with ample translucent germinal tissue (follicular tag)	4	3
Anagen/Catagen bulb only (no translucent tissue present)	4	3
Anagen/Catagen with bulb and translucent sheath tissue	0	0
Total	17	6

retrospective study used the grinding method of extraction discussed herein.

AmpliType PM + DQA1 Typing of Head Hair Roots

Discussion

Half of the telogen club hairs used in this study had the "cotton swab" root end appearance and no surrounding translucent tissue present. It is possible to obtain mtDNA product from this type of late telogen hair by using the grinding extraction method for PCR amplification (4). No appreciable difference has been seen however in the amount of mtDNA obtained from hair shafts with attached telogen root clubs versus 2-cm hair shaft pieces with no root (personal communication, J.A. DiZinno, FBI, April, 1997).

The hair examiner should recognize that the telogen root club is fairly clear in whole mount light microscopy. The dark microscopic air vacuoles in the club should not be interpreted as pigment clumps (Fig.(s) 3A, 3B). Pigmentation does accompany the DNA rich anagen hair bulb (Fig.(s) 4A, 4B).

Hairs with evenly pigmented bulbs (catagen/anagen) present showed a high degree of typability due to the presence of the DNA-rich dermal papilla and surrounding intact matrix bulb cells. This structure can be incorrectly identified and therefore underestimated for DNA content by experienced microscopists as a telogen club (no usable nuclear DNA), since no translucent surrounding tissue may be present. This study and review of prior casework DNA hairs showed that telogen hairs with ample translucent germ cell/epithelial tissue (follicular tag) were easily typable by PCR methods (Fig.(s) 6C, 6D).

Hairs with anagen bulbs and inner/outer root sheath shaft tissue, in varying states of degeneration, will usually produce a large amount of nuclear DNA PCR amplified product for obvious reasons. Found hairs with unusually large amounts of tissue present within the bulb and on the shaft are candidates for restriction fragment length polymorphism (RFLP) analysis. Cases microscopically identified for such analysis should not be extracted with Chelex 100 because that procedure results in a denatured DNA sample unsuitable for RFLP (13). It is worthy to note that no probative evidence hair with anagen/catagen bulb and translucent root sheath material has been submitted for DNA analysis in the last eight years by the Dallas County Institute of Forensic Sciences. Hairs with anagen bulbs or early catagen bulbs and no translucent peripheral tissue, should be considered very good candidates for PCR DNA amplification. It is possible that these types of hairs were referred to as "shed" or "combed" in earlier reports (1,5). Hairs microscopically identified as in the late telogen growth phase without abundant translucent germ/epithelial cell tissue produced no PCR amplified nuDNA product in this study. It is noted that DNA concentration and DNA quantification was not attempted after extraction.

A resinous mounting medium with a refractive index (R.I.) similar to that of the cuticle of hair is used to reduce the diffraction of light at the hair edges in whole mount preparations (Permount has an R.I. of 1.525 at 25°C). Optical clarity is necessary for the microscopic comparisons and root end photographic documentation. These authors do not agree that hairs can be microscopically compared without suitable mounting medium prior to DNA analysis as reported by Hochmeister, et al. (3). The forensic examiner usually has to microscopically examine numerous hairs from a crime scene before a possible suspect hair suitable for tedious comparison is found.

Grinding of hair roots and hair shafts is the optimal method for obtaining mitochondrial DNA (4). No glass grinder cross contamination or other sources of PCR contamination were detected in this study which was limited to nuDNA typing. The same grinder pestal and tube was used for 4–5 hairs and discarded. The ground glass inner surface of the tube is exhausted after about 4–5 hairs have been individually homogenized.

Conclusions

Findings from this study were generally consistent with a 1981 study by Hukkelhoven, et al., which detailed DNA content found in the hair sheath, elongation zone, and bulb using a fluorimetric microassay technique (14). The most DNA was found in the translucent epithelial sheath tissue adhering to the anagen head hair shaft above the elongation zone (stem) and bulb. The pigmented anagen/catagen elongation zone (stem) contained the next best typable DNA, followed by the successful typing of the anagen/catagen hair bulb alone. The 1981 study only examined "freshly plucked head hairs with visible bulb and sheath material." The 1988 R. Higuchi, et al., report used the terms "freshly plucked" hairs and "shed" hairs to report a maximum recovery of 200 ng of DNA from the former and a maximum recovery of 10 ng of DNA from the latter (1). The microscopic appearance of the root of the "shed" hairs was not reported. It is doubtful that the "shed" hairs had telogen clubs or telogen clubs with germ nipples. It is unlikely that the "combed" hairs reported in the FISH study had telogen clubs only (5). In fact, one of the authors of this manuscript (JAP) reports that hair type (telogen vs. anagen/catagen) was not recorded in that study. Uniformity in nomenclature is strongly urged. With practice, one can microscopically identify a single hair's particular growth stage in light microscopic whole mounts. Forensic hair examiners prefer to report the terms anagen and telogen, rather than "plucked and shed" or "pulled and combed." The catagen growth phase is rarely reported because of its short duration (2–4 weeks) and ambiguous whole mount microscopic appearance (6). All hairs with roots submitted to the laboratory could be classified as telogen with club, telogen with germ nipple, telogen with ample germ tissue, anagen/catagen bulb with a root sheath, and anagen/catagen bulb without root sheath tissue (Fig. 1).

The anagen hair bulb, which is evenly pigmented, has numerous viable cells (matrix and melanocytic) with intact nuclei, and contains the DNA rich dermal papilla, should not be confused with the telogen club, which is not pigmented, has no identifiable cells, and has a macroscopic cotton swab appearance. During catagen the bulb shrinks, setting the dermal papilla free. The lower follicle consists of a thin cord of epithelial cells surrounded by the fibrous root sheath. The thin cord of epithelial cells retract upward forming a small nipple attached to the newly formed club. This is referred to as the secondary hair germ nipple tissue. This is the onset of telogen (8). Many examiners use the term "follicular tag" to describe the abundant secondary translucent germ tissue attached to a found telogen hair club (15).

The telogen club is formed after the transformation of the outer root sheath into germ cells which encapsulate the club with a sac. The telogen club rises toward the skin surface while there is shrinkage and descent into the skin by the bulb and its adherent collapsed fibrous structures. The telogen club is simply a product of apoptosis (cell deletion) and keratinization (8). The previous anagen/catagen root structures are not discernable at this point and the overall hair root length is decreased by two-thirds through cell deletion (apoptosis) (8). This is the type of hair resting in the scalp waiting to be naturally shed or forcibly plucked. In the absence

of translucent root sheath material, an experienced hair examiner may confuse telogen club and anagen bulb structures and therefore lack judgment about a found hair's potential DNA content. The translucent germ nipple tissue often accompanies the early telogen head hair upon removal (Fig. 1E). The usable nuclear DNA content of this nipple structure is minimal. There are forensic literature reports which confuse the use of the terms "club" and "bulb." A bulb is a viable structure at anagen/catagen root ends and a club is the nonviable structure at the late telogen hair root end.

Few mitochondria were identified in the hair bulb matrix or cortical cells of this study by transmission electron microscopy (Fig. 5E). Mitochondria are difficult to demonstrate in hair root cells. Poor penetration of TEM fixative through tonofilament/keratin filament material and the masking of mitochondria by trichohyalin granules and melanosomes are a few reasons for this (16). When small pieces of skin with hairs are fixed and sectioned, a few mitochondria can be seen near the nucleus of matrix cells in the anagen hair bulb. Mitochondria are more numerous in the root upper bulb where cells are larger. Mitochondria are seen more frequently in the epidermal telogen club epithelial sac (follicular tag) than in the telogen hair germ nipple tissues (16).

This laboratory does not attempt to microscopically associate found limb hairs to individuals. These hairs are indistinctly pigmented and do not offer the examiner a "pigment pattern" from which meaningful associative judgments can be made. Limb hairs are simply reported of possible arm or leg origin. Although the anagen phase of limb hair growth is only several months long, the same root end morphologic descriptions are applicable when considering DNA PCR amplification of that material (6). This laboratory does include found pubic hairs, along with head hairs, as candidates for microscopic comparison to known pubic and head hair standards respectively. Pubic hair roots can be evaluated for the same morphologic structures described here for determining PCR DNA potential, although pubic hair periods of rest (telogen) are longer than their periods of growth (anagen) (16). Anagen pubic hairs are found less frequently than telogen pubic hairs with ample translucent germ cell tissue on physical evidence items (Fig. 6).

Because evidence hairs can become contaminated with foreign body fluids and skin cells, the hair microscopist must be able to correctly distinguish all root structures from foreign material in order to defend contamination issues in the courts when DNA PCR typing has been done. Exact determination of anagen versus catagen growth stage is not always possible in resinous whole mount preparations. Potential DNA containing root structures can always be correctly identified. The amount of innate nuclear DNA obtained from a hair root is an indicator of that hair's particular growth phase when it left the scalp.

Observations reported in this paper regarding hair roots and their nuDNA PCR typability are the result of this study and a review of eight years of casework. Hair nuDNA PCR typing results that were completed by several different laboratories for Institute of Forensic Sciences cases were reviewed. Findings from this study and the retrospective study indicate that evidence hairs do not appear to be compromised by mounting in Permount prior to nuclear DNA amplification. (Telogen club hairs and telogen club hairs with germ nipples not placed in Permount also produced no nuDNA product upon PCR amplifications.)

The focus of forensic biology on DNA typing methods has led to a gradual decline in hair microscopy practice and training. The cost of indiscriminate DNA analyses without benefit of a meaningful microscopic assessment of the hair goes beyond the obvious financial waste to the equally critical waste of analytical time. For those circumstances in which the victim and the offender paths did not previously cross, the microscopic examination of hair remains strong associative evidence.

These authors urge conformity among researchers and examiners when describing a particular hair root type. Pulled, plucked, combed and shed descriptions of hair roots are misleading. Terms such as anagen/catagen bulb with sheath, anagen/catagen bulb with no sheath tissue, telogen club with ample germinal tissue (follicular tag), telogen club with germ tissue nipple and telogen club are more appropriate terms (Fig.(s) 1 and 6). Some forensic examiners use the terms club and bulb interchangeably. These structures are very different, macroscopically, ultrastructurally, and functionally.

The following is a summary of the conclusions presented herein: (1) FISH does not appear to be a useful forensic test for routine incorporation into hair examination. (2) Microscopic hair evaluation and comparison using a suitable mounting media should always precede DNA testing. (3) Hair examiners should use appropriate (and consistent) dermatologic terminology when describing hair root morphology. (4) Telogen hair clubs should not be submitted for nuDNA PCR typing. (5) Anagen/catagen hair bulbs absent translucent sheath tissue are excellent candidates for nuDNA PCR typing.

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