2018

A Review and Conceptual Model of Factors Correlated with Postmortem Root Band Formation

Joseph Donfack
Joseph.Donfack@ic.fbi.gov

Hilda S. Castillo
Oak Ridge Institute for Science and Education

JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, 2018
doi: 10.1111/1556-4029.13776
Available online at: onlinelibrary.wiley.com

CRITICAL REVIEW

CRIMINALISTICS

Joseph Donfack,[1] *Ph.D.; and Hilda S. Castillo*,[2] *Ph.D.*

A Review and Conceptual Model of Factors Correlated with Postmortem Root Band Formation*,†

ABSTRACT: It is generally accepted within the forensic trace evidence community that a postmortem root band (PMRB) can appear in the root of hairs attached to remains during decomposition. Presently, the specific sequences of events and/or exact molecular signals that lead to the formation of a PMRB are not well understood. The published literature addressing the abiotic and biotic factors that correlate with the formation of PMRBs is reviewed and a conceptual model for the formation of PMRBs is proposed.

KEYWORDS: forensic science, forensic hair analysis, microscopy, trace evidence, hair root, postmortem root band (PMRB)

Definition of Terms: The following terms are defined for clarification purposes.

- *in situ* postmortem head hair: hair attached to the scalp of a human decedent
- *ex situ* postmortem head hair: hair plucked from the scalp of a human decedent
- *ex situ* antemortem head hair: hair plucked from the scalp of a living human
- postmortem root band (PMRB): dark ellipsoidal band (when viewed in transmitted light) appearing in the prekeratinized region of a plucked *in situ* postmortem head hair
- antemortem root band (AMRB): dark band variable in shape (when viewed in transmitted light) appearing in the prekeratinized region of an ex situ antemortem head hair that has been exposed to specific experimental conditions.

[1]Counterterrorism and Forensic Science Research Unit, Federal Bureau of Investigation Laboratory Division, 2501 Investigation Parkway, Quantico, VA 22135.

[2]Visiting Scientist Program, Oak Ridge Institute for Science and Education, Oak Ridge, TN 37830.

*Supported in part by an appointment to the Visiting Scientist Program at the Federal Bureau of Investigation (FBI) Laboratory Division, administered by the Oak Ridge Institute for Science and Education, through an interagency agreement between the US Department of Energy and the Federal Bureau of Investigation.

†This is publication number 16–36 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for information only and inclusion does not imply endorsement by the Federal Bureau of Investigation or the U.S. Government. The views expressed are those of the authors and do not necessarily reflect the official policy or position of the Federal Bureau of Investigation or the U.S. Government.

Received 26 Dec. 2017; and in revised form 6 Feb. 2018; accepted 10 Feb. 2018.

The root of a human anagen or early catagen hair embedded in scalp tissue can undergo decompositional changes following death (1–3). One example of such a change was initially discussed in 1984 by Seta et al. as a darkening around the root end of hair collected from a cadaver (2). Four years later in 1988, this microscopic feature of hair decomposition was further characterized and defined as "an opaque ellipsoidal band composed of a collection of parallel elongated air/gas spaces and is approximately 0.5 mm above the root bulb and about 2 mm below the skin surface" by Petraco et al. (1). For the first time, the described hair feature was referred to as a "postmortem root band" (PMRB). The enclosed air/gas in a PMRB is responsible for the appearance of the banded area as dark in transmitted light and bright in reflected light. In 2005, the Scientific Working Group for Materials Analysis (SWGMAT) and the European Network of Forensic Science Institutes (ENFSI) revisited PMRB and defined it as "the appearance of an opaque microscopic band near the root area of hairs from a decomposing body" (4) and "an opaque microscopic band that can be observed near the root area of hairs from a decomposing body" (5).

Postmortem hair root banding is a recognized phenomenon within the forensic hair community. It is generally accepted that it can appear in the root of hairs attached to remains during decomposition (4–6). However, the specific causative factor(s) and mechanism(s) that influence the formation of a PMRB are not well understood. Despite the recognized gaps in our knowledge of PMRBs, the number of studies exploring this hair feature has only recently increased.

Numerous publications describing the structure, growth, and biochemical composition of hair in great detail are available (7–11). The time it takes a PMRB to appear was discussed extensively by Kadane (12). These topics will not be revisited here. Instead, this review is a comprehensive analysis of relevant studies specifically addressing the factors that correlate with decompositional changes of hair and the formation of PMRBs. Emphasis was placed on experiments investigating the formation

of PMRBs. The factors were categorized as follows: (i) temperature, (ii) environment, (iii) microbial and/or enzyme activity, (iv) autolysis, and (v) degradation of the intermacrofibrillar matrix (IMM). It is important to note that some of these factors may not operate independently.

Temperature

At least two studies explored the effect of temperature on the formation of PMRBs. In 1996, Collins (13) published a study in which small sections of human cadaver scalps bearing *in situ* postmortem head hairs were exposed to three different indoor temperatures; −70°C, 4°C, and 20°C (room temperature). The exact number of hair specimens and/or human cadavers was not discussed in detail. Approximately every 24 h for up to 192 h, 5–10 *in situ* postmortem head hairs were plucked from each scalp section. To assess the level of decompositional changes occurring over time, each collected hair root was individually mounted on a microscope glass slide using Xylene and Permount® Mounting Medium and examined via light microscopy. Collins found that negative control hair samples (i.e., hairs plucked and examined prior to allowing scalp sections to decompose at the different temperatures) and *in situ* postmortem head hairs stored at −70°C did not exhibit PMRBs. However, *in situ* postmortem head hairs stored at room temperature exhibited early signs of PMRB formation manifested by the appearance of thin light bands at 24 h, and fully formed PMRBs manifested by the appearance of thick dark bands at 72 h. *In situ* postmortem head hairs stored at 4°C did not exhibit the same level of band thickness and darkness as those stored at room temperature. This study demonstrated that temperature is a major factor affecting both the formation and progression of PMRBs, with higher temperatures accelerating the process.

In 2013, Koch et al. (6) published a study in which they examined 20,007 *in situ* postmortem head hairs obtained from 23 human cadavers allowed to decompose at different temperatures at the University of Tennessee's Forensic Anthropology Center. *In situ* postmortem head hairs were plucked daily from various areas of each cadaver's scalp. To assess the level of decompositional changes occurring over time, a sampling of hairs from each collection area was mounted on microscope glass slides using Permount® Mounting Medium and examined via light microscopy. It is important to note that Koch et al. (6) evaluated several experimental conditions, but only the three showing the effect of temperature on PMRB formation are discussed in this section. The first experimental condition involved maintaining the cadavers inside a low temperature-controlled building for up to 34 days during the month of January. The average daily indoor temperature was ~5.8°C. The second condition involved maintaining the cadavers in the interior of stationed vehicles (including trunk) for up to 4 days during the month of August. The average daily temperature in Tennessee during that particular month and year was ~26°C. The third condition involved maintaining the cadavers in the interior of stationed vehicles (including trunk) during the month of January for up to 31 days. The average daily temperature in Tennessee during that particular month and year was ~3°C. PMRBs first appeared in hairs from cadavers maintained in the interior of stationed vehicles (including trunk) after 4 days in August and after 12 days in January, but was delayed for up to 14 days for hairs from cadavers maintained inside a low temperature-controlled building. The relatively low and constant indoor temperature seemed to slow or prevent the formation of PMRBs. Additionally, Koch et al. (6) found that the rate of PMRB formation and progression was greater for hairs collected during August, compared to that of those collected during January. In agreement with Collins' work (13), this study demonstrated that temperature positively correlates with the level of decompositional changes observed in the examined hair roots. Overall, warm temperatures accelerate and controlled cool temperatures delay the formation of PMRBs.

A multitude of factors can affect the decomposition process of *in situ* postmortem head hair roots. The above two studies clearly demonstrate that temperature is one of them. However, just because there is a correlation between temperature and the formation of PMRBs, this does not necessarily mean that one causes the other. Temperature alone is unlikely to be the causative agent of PMRBs.

Environment

The effect of environment on the formation of PMRBs is, by far, one of the most assessed factors. Based on an analysis of relevant literature, the different environments investigated include: soil (outdoor soil, indoor fresh soil, and indoor potting soil), sand (indoor), water (freshwater, pond water and saltwater), ambient air (indoor and outdoor in wooded areas), and stationed vehicles (interior and trunk).

In addition to exploring the effect of temperature on the formation of PMRBs as discussed earlier, Collins (13) also investigated the effect of environment. She exposed small sections of human cadaver scalps bearing *in situ* postmortem head hairs to two different environments; on the surface or buried a few inches below indoor soil or indoor sand. Again, every 24 h for up to 8 days, 5–10 hairs were collected and examined as described previously. Hairs exhibited fully formed PMRBs at 72 h, with no differences in formation or progression observed when comparing soil and sand samples.

Unlike Collins' study during which *in situ* postmortem hair samples were used (13), in 2001, Linch and Prahlow (14) described several experiments performed by one of their collaborators using two sets of hairs plucked from the scalp of living humans. These hairs will be referred to as *ex situ* antemortem hairs. The exact number of hair specimens and/or human donors was not discussed in detail. The first set of hairs was exposed above ground to outdoor ambient air in a wooded area for 42 days during the months of January and February. The average daily temperature in California during those particular months and year was not specified. The second set was exposed to the same outdoor wooded area for 14 days during the months of July and August. The average daily temperature in California during those particular months and year was ~38°C. To assess the level of decompositional changes occurring over time, each hair root was mounted on a glass slide and examined via light microscopy. The frequency of hair sampling for microscopical analyses and the type of mounting medium used were not provided. No apparent changes were observed for any of the hairs examined (14). These results suggest that ambient conditions alone do not lead to advanced decompositional changes in *ex situ* antemortem head hairs.

In 2004, Domzalski (15) presented work detailing the microscopic changes of *ex situ* antemortem head hairs obtained from four human donors. She designed five experiments each using sets of 3 or 13 anagen or telogen head hairs which were exposed to differing indoor environments for the time periods ranging from 8 days to 7 months. The environments evaluated included: airtight

setting (negative control), fresh soil burial (indoor potting), pond water immersion, and ambient air. Two of the five experiments described by Domzalski (15) were designed to explore the effect of sterile indoor environments on the formation of a PMRB and will be discussed in the "microbial and/or enzyme activity" section. The average daily indoor temperature was ~22°C. To assess the level of decompositional changes occurring over time, a sampling of hairs from each indoor environment was individually mounted on microscope glass slides using Histoclear™ (for temporary slide mounts) or Cargille™ Meltmount (for permanent slide mounts) and examined via transmitted, brightfield light microscopy. The frequency of hair sampling for microscopical analyses depended on the experiment number and varied from 1 to 8 different time points during which, several observations were made. First, negative control hair samples stored in airtight settings did not exhibit PMRBs. Second, *ex situ* antemortem telogen hairs (resting phase) exhibited fewer to no changes compared with *ex situ* antemortem anagen hairs (active growth phase) kept in the same soil and water environment. Third, anagen hairs immersed in soil or water exhibited more changes in hair root morphology (including band formation) compared with anagen hairs kept in ambient air. Overall, Domzalski's work showed that less keratinized layers of the hair root are more susceptible to degradation compared with fully keratinized layers (this finding was initially reported by Petraco et al. [1]), and that humid and aqueous conditions exhibited strong positive correlations with hair root band formation. In agreement with the experiments described by Linch and Prahlow (14), ambient air alone does not lead to advanced decompositional changes in *ex situ* antemortem head hairs. Domzalski reported that antemortem root banding (AMRB) can be observed in *ex situ* head hair samples exposed to certain environments. Additionally, Domzalski noted a difference in location between AMRBs and PMRBs with respect to the root end; AMRBs being more proximal than PMRBs.

Eight years later (2012), Shaw et al. (16) also contributed to our understanding of the effect environment has on the formation of hair root banding. Approximately 600 *ex situ* antemortem head hairs were collected from 15 human donors. Sets of 25 hairs were placed in differing environments in the Northern Virginia area for time periods ranging from 7 days to 7.5 months between August 2010 and April 2011. The environments evaluated include: outdoors (above ground protected and unprotected from sunlight), indoors (windowsills, fresh/well water immersion, and potting soil burial), and stationed vehicles (front dash and trunk). The average daily temperature in Northern Virginia during those particular months and years ranged from ~8°C to ~37°C. When the level of decompositional changes occurring over time was assessed daily via light microscopy, hairs kept in any of the indoor or vehicle environments ($n = 250$) did not show signs of decomposition. In agreement with the works of Linch and Prahlow (14) and Domzalski (15), these results suggest that exposure to ambient air alone does not lead to AMRB formation. Among the samples that did exhibit decompositional changes including AMRB (outdoors [$n = 250$], fresh/well water [$n = 50$] and potting soil [$n = 50$]), the greater percentages of decomposition were observed in hairs buried in potting soil ($n = 39$, 78%) or immersed in water ($n = 44$, 88%). This is also in agreement with Domzalski's work (15). Based on a blind study, the authors also reported that the characteristics of AMRBs are distinguishable from those of PMRBs. The blind study consisted of 208 hairs, with each exhibiting a PMRB or AMRB. Trained trace evidence examiners were able to differentiate PMRBs from AMRBs 99.5% of the time. When quality assurance practices requiring verification by a second examiner were implemented, the accuracy of the study improved to 100%. These findings clearly suggest that even if the formation and progression of PMRBs and AMRBs are similar or the same, other factors (e.g., hair root attachment to decomposing remains) lead to similar yet distinctive microscopic features.

In addition to exploring the effect of temperature on the formation of PMRBs as discussed previously, Koch et al. (6) also investigated the effect of environment. Briefly, the human cadavers used were allowed to decompose in varying environments (outdoors on the surface of soil, in graves of varying depths, in freshwater, and enclosed within vehicle trunks), while also being exposed to a wide range of temperatures. The *in situ* postmortem head hairs were collected and examined daily as described previously. Overall, this study showed that there is a complex network of variables involved in the decomposition of hair. Koch et al. concluded that it was difficult to identify a precise relationship between the rate of hair decomposition and environment, temperature or time (6). Nevertheless, the study provided additional support that decomposition of the proximal end of hair leads to PMRB formation.

In 2013, Delgado (17) published a study seeking to replicate postmortem characteristics in antemortem hairs. A total of 588 *ex situ* antemortem (anagen and telogen phase) head hairs were collected from 14 human donors and exposed to seven different environmental conditions between the months of March and May in 2013. The conditions were saltwater, freshwater, surface of soil, buried within soil, surface of concrete floor, vehicle trunk, and environmental chamber. The average daily temperature in California during those particular months and year was not specified. However, the environmental chamber temperature and humidity levels were set to 28.8°C and 77%, respectively. To assess the level of decompositional changes occurring over time for up to 3 months, each hair root sample was individually examined on a flexible schedule via light microscopy. Delgado found that anagen hairs exposed to water (salt and fresh) and soil (surface and buried) exhibited AMRBs or brush-like hair root ends (i.e., hairs exhibiting continued root decomposition characterized by fraying at the proximal end) (14). However, telogen hairs exposed to these same conditions were resistant to decomposition. This agrees with Domzalski's work (15).

Because the environmental factors described thus far were unable to fully account for the formation of PMRBs, additional answers were sought via *in vitro* studies. In 2015, Hietpas et al. (18) published results from a study controlling the chemical composition of solutions to which *ex situ* antemortem anagen head hairs were exposed (18). Briefly, hair samples from one human donor were trimmed to approximately 1 cm from the proximal end. Each hair was subjected to different experimental conditions (e.g., pH series, protease digestions, and buffer solutions) for up to 7 days at room temperature. The level of decompositional changes occurring over time was assessed daily by mounting each hair root on a microscope glass slide using Xylene and examined via light microscopy. Fleming et al. (19) presented a similar study, but instead used *ex situ* postmortem anagen head hairs. Fleming et al. (19) followed the experimental conditions used by Hietpas et al.'s (18), but immersed 30 hairs in each of the solutions tested for up to 24 days. These two studies showed that decompositional changes including PMRB-like characteristics can be replicated in hairs immersed in the solutions tested with pH ranging from 6 to 8. However, the highest percentage (~70–95%) of decomposition was observed for hairs immersed in a 100 mM ammonium acetate solution (pH 7–8), suggesting that

ammonium and ammonia, often produced in large quantities during decomposition of human remains, are possible chemical agents contributing to the formation of PMRBs (18). If ammonia and ammonium are to be considered as potential causative agents, the natural production of this gas/salt in living humans has to be taken into account as well. Indeed, evidence for a constant diffusion of ammonia from the skin surface into the ambient air was provided in a recent study by Reuther et al. (20). Their work suggests that ammonia is likely produced during keratinocyte (predominant cell type in the epidermis) differentiation known as cornification, because skin and hair undergo very similar differentiation processes. If this is the case, one would expect hairs of living humans to occasionally exhibit signs of root banding. However, no reports of this have been made thus far. One possible explanation for this is that living human cells are metabolically active and equipped with a mechanism that allows ammonia gas to be eliminated into the ambient air, preventing its entrapment between the hair macrofibers and a pull apart of the microfibers to create the parallel and elongated gas spaces associated with hair root banding. It is noteworthy that an attribute of a hair disorder called pili annulati is the closest thing to hair root banding that has been observed in living humans (21). The hair shafts of individuals affected by pili annulati exhibit alternating segments of light and dark banding when examined in reflected light. This hair defect is easily detected in people with blond hair compared to those with dark hair, and it is believed to be the result of abnormal keratinization in the hair shaft. Just as with PMRBs, the bands associated with pili annulati are air pockets within the cortex of the hair shaft (21).

Most recently, Roberts et al. (22) collected 1,002 *ex situ* antemortem (485 anagen and 517 telogen) head hairs from 25 human donors. The hairs were exposed to controlled and uncontrolled experimental conditions for 30–63 days. The controlled conditions included: Nanopure™ water, saline solution (0.9% NaCl), hydrogen peroxide solution (3%), talcum powder, and dehydrated soil. The uncontrolled conditions included: ambient air on a shower floor and buried soil. The daily indoor temperature for the controlled conditions varied from ~10 to ~29.4°C. To assess the level of decompositional changes occurring over time, hairs from each environment were mounted on microscope glass slides using Cytoseal™ 60 Mounting Medium and examined via plane-polarized transmitted light microscopy. Roberts et al. (22) found that 0% of the telogen hairs and 14% of the anagen hairs exhibited root morphology characteristics that resemble those reported in PMRBs. In agreement with previous research (15,17,18), this study showed that it is possible to generate hair root banding in *ex situ* antemortem anagen hairs when they are exposed to different environmental conditions. All the studies published in an effort to understand the influence various environments have on the formation of PMRBs share one thing in common. They do not lead to the identification of the specific biological and/or chemical agent(s) present in the environments that contribute to this hair feature. One potential agent, which has been investigated, is microbial activity.

Microbial and/or Enzyme Activity

As reviewed previously, in 1996, Collins (13) demonstrated that temperature is a major factor affecting the formation and progression of PMRBs. She also hypothesized that the underlying mechanism for PMRB formation may be hair root decomposition (13). Decomposition is a natural process involving the degradation of soft tissues via a myriad of enzymes produced by microbes, as products of their metabolism. While developing her hypothesis, Collins considered microbe access to the prekeratinized region of *in situ* postmortem head hairs. She emphasized that immediately after death; microbes invade the external skin surface of cadavers. However, microbes may not easily breakdown the hair shaft region above the skin surface because it is fully keratinized. Over time, microbial invasion may proceed downward along the hair and toward the hair root. The first vulnerable hair region microbes would have access to is the prekeratinized region. At this point, the digestive enzymes constantly secreted by microbes would degrade the biomolecules present in the vulnerable prekeratinized region, resulting in the production of air/gas that becomes entrapped between the hair microfibers. Microscopical characterization of known PMRBs by Hietpas et al. (23) recently showed that the hypothesized beginning or the proximal end of PMRBs coincides "well with the location where the cuticle layer of the hair is first recognized as a distinct structure". If Collins' microbe hypothesis is true, Hietpas et al.'s work initially appears to suggest that the digestive action proceeds from the outside to the inside. However, their work also shows that the banded regions are limited to the cortex of hairs with no observable damage on the cuticle (23).

Another hypothesis of microbial activity as a cause of PMRB formation comes from Linch et al.'s work (24). In 1998, Linch et al. (24) published a study in which the main goal was to determine whether the fluorescent *in situ* hybridization (FISH) technique could help forensic examiners predict which type of root ends may produce successful DNA profiling results. They also performed a limited *in vitro* experiment seeking to enzymatically create AMRBs. *Ex situ* antemortem anagen head hairs were immersed in a proteinase K solution [20 mg/mL] for 4 h at 56°C. The exact number of hair specimens and/or human donors was not discussed in detail. To assess the level of decompositional changes, each hair root was individually mounted on a glass slide and examined via light microscopy. The type of mounting medium was not provided. Darkening at the root ends was observed, and a photomicrograph of a produced AMRB was presented (24). However, this hair band (like other AMRBs described previously) does not appear to exhibit the typical morphology of a PMRB. Nevertheless, these findings suggest that proteases may play a role in the formation of PMRBs.

Based on an additional study in 2001, Linch and Prahlow (14) reported that PMRBs are often observed via light microscopy in *ex situ* postmortem head hair shafts, in the area where the sebaceous gland enters the hair follicle. According to Linch and Prahlow (14), this observation suggests that the sebaceous gland may be responsible for band formation at least in the hair shaft. This is in disagreement with the hypothesis made by Collins (13), Linch et al. (14), and Hietpas et al. (23). This again suggests that more than one mechanism exists for the formation of PMRBs, and which mechanism takes place or dominates may be determined by a given, specific set of conditions. The sebaceous gland contains enzymes capable of digesting cell components, and it is possible that after death the contents of the gland are released, flowing downward and toward the root, and contributing to the formation of distal PMRBs as opposed to proximal PMRBs.

As reviewed previously, in 2004, Domzalski (15) presented work detailing the microscopic changes in *ex situ* antemortem human anagen head hairs exposed to three different indoor environments. Pond water and soil are environments where microbes can thrive, as opposed to the ambient air environment. The nonsterile and sterile (via autoclave) hairs were exposed to nonsterile and sterile (via autoclave) versions of these environments, to

determine whether the microbes in these particular environments were the causative agents of the decompositional changes observed in the prekeratinized region of the roots. Microscopical examination revealed advanced decompositional changes, including AMRBs. These changes appeared earlier (2 days) in nonsterile hairs exposed to the nonsterile environments, compared to sterile hairs exposed to the sterile environments (8 days). Domzalski (15) concluded that microbial activity partially accounts for the mechanism underlying PMRBs in uncontrolled environments.

These studies clearly suggest that microbes and enzymes play important roles in the formation of PMRBs. However, the identity or general type of these microbes and enzymes was not investigated. Bacteria are often credited as a major driving force for the process of decomposition, but few studies cataloging the microbiome of decomposition have been published. Notably antithetical, Hietpas et al. (23) reported that no evidence of bacteria was observed inside the banded area of hair roots during examinations via light and scanning electron microscopy. This again suggests that more than one mechanism is responsible for PMRB formation depending on the given set of specific conditions.

Autolysis

The research studies previously summarized focus on investigating extracellular factors that may affect the formation of PMRBs. However, some researchers have considered internal factors intrinsic to the keratinocytes. In addition to microbial action, autolysis has been proposed by Petraco et al., (1) and Linch and Prahlow (14) as an underlying mechanism. Although empirical data to support this hypothesis were not provided, it is possible that immediately after death, enzymes, predominantly hydrolytic enzymes from within the differentiating keratinocytes lead to self-digestion (i.e., autolysis) because they are no longer under cellular control. These autolytic enzymes are likely to target vulnerable nonkeratinized cellular components and debris from keratinocyte differentiation such as the IMM and may ultimately result in the production of air/gas that becomes entrapped between the hair microfibers. This theory for the formation of PMRBs is very interesting because it does not rely on the activity of microbes and as a result would be consistent with Hietpas et al. (23) not finding evidence of microbes inside the banded area of hair roots. Additionally, there may be more than one mechanism leading to the formation of PMRBs. That is, one that depends on external chemical agents produced by microbes and another that relies on chemical agents endogenous to the hair root. The latter can further be supported by the fact that PMRBs can be observed as early as 8 h after death (1).

Degradation of the Intermacrofibrillar Matrix

Hietpas et al. (23) used high-resolution microscopy for insight into the potential cause(s) and mechanism(s) for the formation of PMRBs. Essentially, 15 *ex situ* postmortem anagen head hairs, originally collected from three human cadavers by Koch et al. (6), each displaying a PMRB, were sectioned by microtome and examined by scanning electron microscopy (SEM) to characterize the architectural changes observed in the microenvironment of the prekeratinized region of the hair roots (6). The results from this study show that degradation of the IMM, a nonkeratin and chemically labile structure, may be responsible for the appearance of PMRBs (23). In addition to degradation of the IMM occurring in the prekeratinized region of the hairs, it is confined to the cortex with no apparent damage to the layers of the cuticle. Like other studies previously discussed, Hietpas et al. (23) did not address in detail the specific interactions between all abiotic and biotic factors orchestrating this degradation. However, a very detailed description of the ultrastructural characteristics of PMRBs using microscopical techniques was provided. These authors plan to expand on a possible mechanism(s) for band formation in future communications.

Conclusion

Overall, the collection of reviewed studies focused on investigating the cause(s) and mechanism(s) of PMRB formation. Most suggest that the contribution of multiple factors (e.g., temperature, environment, and microbes) is involved. Progress has been made in understanding the correlations and possible impacts of these factors. However, one major question remains: What serves as the primary cause(s) and mechanism(s) of hair root decomposition and the PMRB formation? Seeking the answer to this question is a challenge, as it requires monitoring PMRB formation (i.e., decomposition of the hair root) in real time and immediately after death. Nevertheless, based on the existing literature, a general model is proposed for PMRB formation as illustrated in Fig. 1. Death is the first step toward the formation

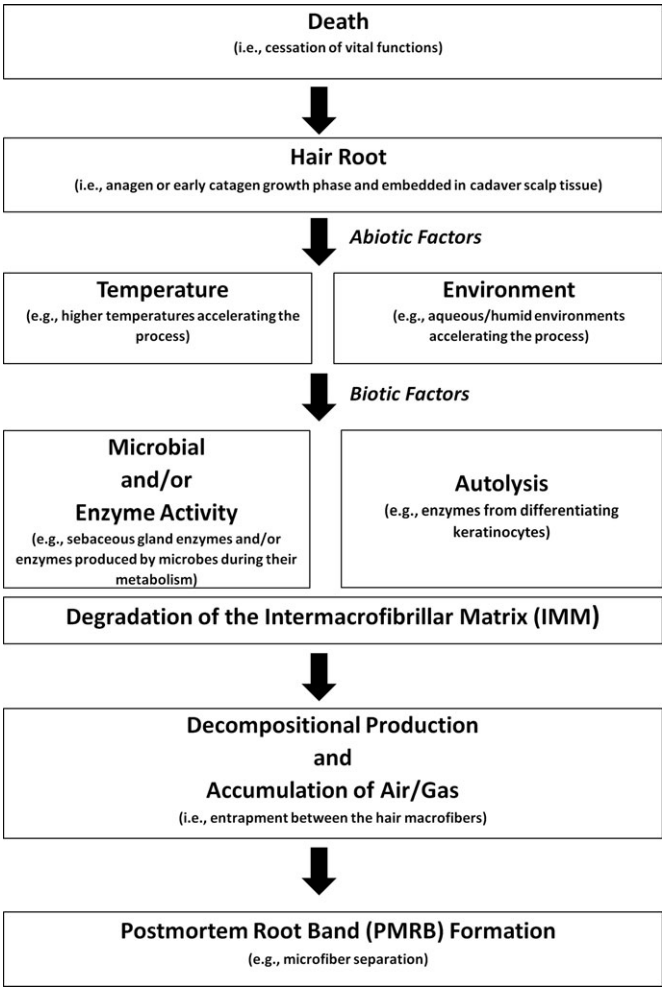

FIG. 1—*A proposed conceptual model of abiotic and biotic factors that correlate with the formation of postmortem root bands (PMRBs).*

of a PMRB. Following clinical death, the human body, including scalp hairs in anagen or early catagen growth phase, undergoes decomposition. Decomposition is well known as a natural process involving a complex network of abiotic and biotic factors. Abiotic factors (e.g., weather, climate, and humidity) can influence PMRB formation. For example, high humidity/aqueous conditions (15–17,22) and warm temperatures (6,13) appear to have a significant effect on the rate of hair root decomposition. Biotic factors (e.g., the intrinsic characteristics of the hair root and microbes/enzymes in the case of PMRBs) have also been proposed to influence PMRB formation (1,14,15,17,23). For example, anagen as opposed to telogen hairs is more susceptible to decomposition (15,17,22). Band formation is, therefore, a function of a specific ecosystem. Variations to the ecosystem, such as someone dying and decomposing indoors versus outdoors, can lead to unique pathways (e.g., microbial and enzyme activity or autolysis) for band formation characterized by the disruption/degradation of the IMM (23). The fact that this multitude of variable factors can overlap and interact is what makes the isolation, and thus identification, of the primary cause(s) and mechanism(s) difficult.

Acknowledgments

The authors would like to thank Linda M. Otterstatter, Karen K. Lowe, and Cary T. Oien for their comments.

References

1. Petraco N, Fraas C, Callery FX, De Forest PR. The morphology and evidential significance of human hair roots. J Forensic Sci 1988;33:68–76.
2. Seta S, Sato H, Yoshino M, Miyasaka S. Morphological changes of hair root with time lapsed after death. Conference abstracts. J Forensic Sci Soc 1984;24:283–457.
3. Tafaro JT. The use of microscopic postmortem changes in anagen hair roots to associate questioned hairs with known hairs and reconstruct events in two murder cases. J Forensic Sci 2000;45:495–9.
4. Scientific Working Group on Materials Analysis (SWGMAT). Forensic human hair examination guidelines. Forensic Sci Commun 2005;7:1–35.
5. European Network of Forensic Science Institutes (ENFSI). Best practice manual for the microscopic examination and comparison of human and animal hair; http://enfsi.eu/?s=Best+practice+manual+for+the+microscopic&post_type= (accessed January 2018).
6. Koch SL, Michaud AL, Mikell CE. Taphonomy of hair – a study of postmortem root banding. J Forensic Sci 2013;58:S52–9.
7. Birbeck MS, Mercer EH. The electron microscopy of the human hair follicle: part 1. Introduction and the hair cortex. J Biophys Biochem Cytol 1957;3:203–14.
8. Birbeck MS, Mercer EH. The electron microscopy of the human hair follicle: part 2. The hair cuticle. J Biophys Biochem Cytol 1957;3: 215–22.
9. Birbeck MS, Mercer EH. The electron microscopy of the human hair follicle: part 3. The inner root sheath and trichohyaline. J Biophys Biochem Cytol 1957;3:223–30.
10. Lee YJ, Rice RH, Lee YM. Proteome analysis of human hair shaft: from protein identification to posttranslational modification. Mol Cell Proteomics 2006;5:789–800.
11. Popescu C, Hocker H. Hair – the most sophisticated biological composite material. Chem Soc Rev 2007;36:1282–91.
12. Kadane JB. Post-mortem root banding of hairs: a sceptical review. Law Probab Risk 2015;14:213–28.
13. Collins BW. The effect of temperature and environment on post mortem morphology of human hair roots [dissertation]. New York, NY: John Jay College of Criminal Justice, 1996.
14. Linch CA, Prahlow JA. Postmortem microscopic changes observed at the human head hair proximal end. J Forensic Sci 2001;46:15–20.
15. Domzalski AC. The effects of environmental exposure on human scalp hair root morphology. [dissertation]. New York, NY: John Jay College of Criminal Justice, 2004.
16. Shaw SD, Otterstatter L, Shegogue C. The microscopic characteristics of antemortem and postmortem hairs at the root end. Proceedings of the 64th Annual Scientific Meeting of the American Academy of Forensic Sciences; 2012 Feb 18-23; Baltimore, MD. Colorado Springs, CO: American Academy of Forensic Sciences, 2012.
17. Delgado RJ. An investigation to replicate post mortem characteristics in ante mortem anagen head hair [dissertation]. Long Beach, CA: California State University Los Angeles, 2013.
18. Hietpas J, Buscaglia J, Richard AH, Castillo HS, Shaw SD, Drummond EJ, et al. The investigation of potential mechanisms of the formation of postmortem hair root bands: a detailed microscopical and ultrastructural analysis. Proceedings of 67th Annual Scientific Meeting of the American Academy of Forensic Sciences; 2015 Feb 16 - 21; Orlando, FL. Colorado Springs, CO: American Academy of Forensic Sciences, 2015.
19. Fleming JN, Castillo HS, Drummond EJ, Jabbour R, Deshpande S, Steadman DW, et al. *In-vitro* experiments using human cadaver head hairs to investigate the formation mechanism of postmortem hair root bands. Proceedings of 68th Annual Scientific Meeting of the American Academy of Forensic Sciences; 2016 Feb 22 – 27; Las Vegas, NV. Colorado Springs, CO: American Academy of Forensic Sciences, 2012.
20. Reuther T, Shams G, Kerscher M. Evidence for a steady diffusion of ammonia from the skin surface into the environment as gaseous ammonia leaving. J Invest Dermatol 2014;134:S43–52.
21. Ito M, Hashimoto K, Sakamoto F, Sato Y, Voorhees JJ. Pathogenesis of pili annulati. Arch Dermatol Res 1988;280:308–18.
22. Roberts KA, Garcia LR, De Forest PR. Proximal end root morphology characteristics in antemortem anagen head hairs. J Forensic Sci 2017;62:317–29.
23. Hietpas J, Buscaglia J, Richard AH, Shaw S, Castillo HS, Donfack J. Microscopical characterization of known postmortem root bands using light and scanning electron microscopy. Forensic Sci Int 2016;267:7–15.
24. Linch CA, Smith SL, Prahlow JA. Evaluation of the human hair root for DNA typing subsequent to microscopic comparison. J Forensic Sci 1998;43:305–14.

Additional information and reprint requests:
Joseph Donfack, Ph.D.
FBI Laboratory Division
Counterterrorism and Forensic Science Research Unit
2501 Investigation Parkway
Quantico
VA 22135
E-mail: Joseph.Donfack@ic.fbi.gov