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New methods for establishing time of death when dealing with natural mummification from bog environments

by Tristan Mula

Natural mummies are human and animal remains that have been naturally preserved over time. In most cases, these mummies are formed through a combination of environmental factors such as the soil's chemical makeup, temperature, and humidity. One of the most well-known, yet uncommon, examples of natural mummies are those found in bogs, wetland environments characterized by low oxygen levels and acidic water. Mummies discovered in these bog environments will be the focus of this paper. It will discuss the challenges associated with establishing the time of death for natural mummies. Various factors influence the mummification process including the acidity of the water, temperature, and the presence of microorganisms. As a result, traditional methods of estimating the time of death, or post-mortem interval (PMI), may not be reliable. This paper will also review recent advances in the field, including but not limited to stable isotope analysis, DNA sequencing, and proteomics. This will allow researchers to understand the taphonomic processes at play and improve the accuracy of time of death estimations. Overall, this paper provides practical insights into the complex processes involved in determining the time of death in natural mummies and offers information about new technologies useful for researchers in this field.

Naturally formed mummies, not mummified through human intervention, are a fascinating subject of study that provide invaluable information about the diet, health, and daily activities of people from the past. Among the different types of mummies, bog bodies are particularly interesting, due to their unique and rare nature. Bog bodies are formed when a body is buried in a peat bog environment, leading to the exceptional preservation of tissue and skin for centuries or even millennia. The term "bog body" was first introduced by Johanna Mestorf in 1871 to describe twelve sets of well-preserved remains found in Ireland, Denmark, and Germany (Giles 2020).

This paper examines the mechanics of bog body formation and their taphonomic processes, but how did these individuals come to be deposited in bogs? Various theories range from accidental drownings to ritualistic sacrifices and the possibility of executed criminals being interred in these watery graves. Ravn's (2010) paper looks at various bog bodies found in Europe, presenting evidence suggesting that some may have been executed as punishment. Using the characteristics of these bodies, such as signs of violence and the presence of restraints, and analyses of their historical and cultural contexts to support the hypothesis. Ravn (2010) sheds light on these ancient bog bodies' potential criminal and judicial aspects, providing insights into past societal practices and legal systems.

Despite numerous discoveries of bog bodies, there is no standardized protocol for their analysis comparable to the established methods for assessing skeletal material in human osteology, such as those formulated by Buikstra and Ubelaker (1994). This lack of standard methods for analyzing bog bodies poses a significant challenge for researchers, as these remains do not follow the timelines they are familiar with,

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making it challenging to make determinations like the post-mortem interval (PMI) (Wang et al. 2017). Given the unique quality of preservation and the challenges posed by the lack of standardized methods, bog bodies require a tailored approach to their analysis. Therefore, it is crucial to establish a specific protocol for analyzing these remains to assess their PMI and other characteristics. By studying bog bodies, researchers can better understand how people lived, their health, and their practices, and develop a more comprehensive understanding of human history and culture.

Natural Preservation of Human Remains

Understanding the process by which natural mummies are formed is necessary to help forensic anthropologists adjust their methods for establishing a time of death. Natural mummification is a process in which an organism's remains are preserved. This requires spontaneous conditions that allow for quick dehydration, including hot climates, dehydration in freezing climates, or preservation by an anaerobic and acidic environment (Joshi 2021). The specific methods for forming natural mummies can vary depending on the environmental conditions. When an organism dies, and its body is left in a preservation-conducive climate, such as a desert or a tundra, the body will lose moisture through evaporation, which can help prevent decay and the growth of bacteria and fungi (Guthrie 1973). As will be discussed later in more detail, enzymatic activity is a key factor in the decomposition of a corpse. For catalyzation of enzymes, most require an aqueous median (Nielsen et al. 2021). This means when a body is in an environment that provides opportunity for quick dehydration, it can cause the cessation of enzymatic activity before decomposition can occur. The clothes of the deceased may also aid in this process by allowing the water pooling on the epidermis to be wicked away (Piombino-Mascali, Gill-Frerking, and Beckett 2017). If there are signs of decomposition on a mummified corpse, it is usually situated around the torso where the abdomen provides a surplus of enzyme-rich water, or at levels of low gravity where pooling of liquids might have occurred (Piombino- Mascali, Gill-Frerking, and Beckett 2017). The hot, dry environment can mummify an individual in only two weeks while climates that are more temperate can take around three months (Leccia, Alunni and Quatrehomme 2018).

The preservation of the mummies' soft tissue varies depending on the conditions of the burial environment. This can be so significant that even within a small microenvironment, the levels of preservation can vary. This can be seen in the famous Tollund Man from Denmark. In this human sacrifice from around 380 B.C.E, the individual’s head and feet are well-preserved but the arms and hands had been completely skeletonized (Nielsen et al. 2021; Piombino-Mascali, Gill-Frerking, and Beckett 2017). The preservation of soft tissue is also dependent on how the body is situated: if parts of the body are lying on an even surface, they may become flattened, or if a part of the mummy is in contact with another part, there might be some visible decay (Piombino-Mascali, Gill-Frerking, and Beckett 2017). The internal organs of the corpse, if still present, will usually collapse and greatly reduce in size. Aufdherheide (2003) and Wittmer and colleagues (2008) developed a simple quantitative method to help give a score to the amount of soft tissue remaining. This was achieved by assigning a numerical value for the amount of bone, hair, and other bodily components still observed at the thorax, abdomen, and limbs. (Piombino-Mascali, Gill-Frerking, and Beckett 2017).

In some cases, the body may become enclosed or partially enclosed in a material that can further protect it from decay from external factors, such as the prevention of insect activity reaching the body (Leccia, Alunni and Quatrehomme 2018). The longer the body remains in the burial environment, the more likely it becomes preserved as a natural mummy.

The mummification process in bog bodies differs from that of natural mummies formed in dry environments. A bog body is a preserved corpse in wetland peat bog deposits (Piombino-Mascali, Gill-Freking, and Beckett 2017). There are two types of bogs where you may find human remains: the Ombrotrophic peat and the Minerotrophic wetlands also known as fens. The Ombrotrophic peat bogs are much more acidic than the alkaline Minerotrophic wetlands around pH 3.2–5 and pH 5.0–8.0 respectively. The high acidity environment produces excellent preservation of soft tissues (Piombino-Mascali, Gill-Freking, and Beckett 2017). Ombrotrophic peat is primarily found in parts of Northern Europe, but in 1982, burials dating to more than 8,000 years ago were discovered in Florida (Tyson 2006). Known as the Windover burials, 91 of the 168 burials had preserved soft tissues. The wet, acidic, and anaerobic conditions of bogs can slow down the decay of organic matter and create an environment that favors the preservation of soft tissues. In bog environments, the acidic water and lack of oxygen can cause the skin to become tanned and toughened. Simultaneously, the high levels of tannins, important plant life, and other chemicals in the water can help preserve the tissues.

Plant life in bogs is an important driver that allows for such great preservation. An important plant is Sphagnum moss, which grows on the top of bogs. As the plant dies, it sinks toward the bottom of the bog and starts its decomposition process. As the Sphagnum decomposes, polysaccharides are released, known as ‘sphagnum’; the release of ‘sphagnum’ starts chelating possesses (Piombino-Mascali, Gill-Freking, and Beckett 2017). Chelating simply means that the ‘sphagnana’ released attach themselves to the metal ions, creating a water soluble product allowing the metal in the water to move away from the body (Knepper 2003). Metal ions moving away from the body leave bacteria with a lack of nutrients and the bacteria will not be able to multiply and break down the body (Piombino-Mascali, Gill-Freking, and Beckett 2017).

The body will also form adipocere, also known as grave wax or corpse wax, through a natural process that occurs when the body of a deceased human or animal is exposed to moisture and anaerobic conditions. This process involves the transformation of a body's fat into a waxy substance that can help preserve the body and slow down decay and solidify body tissues (Hayman and Oxenham 2020; Tsokos 2005. The transformation of the adipose tissues triggers hydrolysis of triglycerides into glycerin and free fatty acids. This chemical process transforms fats and oils into a soap-like substance through the breaking down of ester bonds and the formation of carboxylic acid and glycerol molecules (Hayman et al. 2020). Initially, it has a waxy texture and a gray-white color, but as the fatty acids crystallize, it becomes more crumbly or solid, leading to the solidification of affected body parts (Tsokos 2005). This substance can mix with other organic compounds to create a gel-like material preserved in the bog for thousands of years. Cultures of the past utilized these ideal bog conditions to preserve foods known as "bog butter" or "bog gum" (Clibborn and O'Laverty 1859).

Water levels of the bogs also play a vital role in determining the amount of preservation. An experiment done by Gill-Robinson (2001) placed piglets within a one-meter square area; the bog environments were almost identical except for the water levels. The piglets that were exposed to high water levels were almost fully preserved while the others that were at a lower water level became fully skeletonized (Piombino-Mascali, Gill-Freking, and Beckett 2017). Moreover, the bodies will remain flexible when mummification occurs in an acidic and anaerobic underwater environment. In some cases, mummies will continue to retain anatomical details such as fingerprints and tattoos (Leccia, Alunni and Quatrehomme 2018). It is imperative that study of these processes continues in order to enhance our knowledge of the preservation of human remains in an archaeological and forensic context.

Natural Mummies and Archaeological and Forensic Contexts

The exceptional preservation of mummies and bog bodies provides unique opportunities for various scientific fields. These specimens can offer insights into mortuary practices, burial rites, and associated artifacts, such as clothing and tools (Giles 2020). Soft tissues also expand the scope of pathological studies, allowing for identifying acute diseases and those that do not affect bone tissue. Furthermore, mummified bodies can reveal body ornamentation, such as tattoos and hair details, providing a more comprehensive understanding of an individual's culture and lifestyle (Lynnerup 2009).

Mummies and bog bodies have also proven valuable in forensic contexts. By studying the preserved soft tissues of these specimens, researchers can identify specific pathogens and better understand ancient disease patterns (Giles 2020). Additionally, mummies and bog bodies can offer clues about causes of death, such as trauma or infection. Moreover, the exceptional preservation of these specimens can help develop and test forensic techniques. For example, researchers have used computed tomography (CT) scanning to create three-dimensional images of mummies and bog bodies, allowing for more accurate and non-invasive analyses of internal structures. This technique has also been used to reconstruct the facial features of individuals, which can aid in identification efforts (Lynnerup 2009).

When a mummified body is discovered in an active forensic case, it is crucial to thoroughly assess the remains to determine the cause and manner of death and the mummy's PMI (Lynnerup 2009). The analysis requires a careful and thorough assessment of all available evidence and a deep understanding of the complex factors that can contribute to the preservation and analysis of mummified remains.

Currently, in forensic contexts, mummified remains are examined on a case-by-case basis (Lennartz 2018). In a recent study that reviewed the autopsies of 20 cases of natural mummies found between 2002 and 2016, researchers developed a new categorization system for the extent of mummification as there was no standardized classification method. The researchers classified individuals with at least 50% of their body skin remaining as having "extensive mummification," and those with 100% body skin remaining as "complete mummies" (Leccia, Alunni and Quatrehomme 2018). As the autopsies describe either mummification or decomposition without consistent terminology, the development of this new categorization system will help provide clarity in identifying and classifying mummified remains. However, the lack of standardization in determining PMI and cause of death for mummy cases remains a challenge and highlights the need for the development of standardized protocols in the forensic examination of mummies.

Decomposition and Post-mortem Interval

One of the first steps, and most challenging in the analysis of bog bodies, is determining PMI. PMI denotes the duration between the discovery or examination of a corpse and the time of death (Byers and Rhine 2017). PMI estimation can provide valuable information for criminal investigations, accident investigations, and identifying remains in missing persons cases (Hayman and Oxenham 2020). Determining an approximate time of death allows investigators to establish timelines, identify potential suspects, and corroborate or refute alibis. In accident investigations, this estimation aids in reconstructing events, which will help to determine if the death resulted from the accident or occurred prior to it (Byers and Rhine 2017). Additionally, PMI estimation plays a vital role in identifying remains in missing person cases by narrowing down the time frame during which the individual went missing, guiding search efforts, and providing critical clues for identification through comparison with antemortem records, dental records, or DNA analysis.

Determining PMI is a complex process that involves evaluating several factors, including body temperature or algor mortis. Once a body's internal temperature matches that of the surrounding environment, it is the beginning of the Algor mortis stage (Joshi 2021), followed by rigor mortis and livor mortis. Rigor mortis occurs when specific chemical changes in a corpse cause the muscles to become stiff. During this process, Adenosine 5′-triphosphate (ATP) is converted to Adenosine diphosphate (ADP), and lactic acid is produced, which lowers the cellular pH—leading to chemical bridges between actin and myosin. This causes the muscles to lock up, resulting in the characteristic stiffness of rigor mortis (Joshi 2021). Livor mortis, also known as post-mortem lividity or hypostasis, is the physical process by which blood accumulates in the blood vessels of the lowest parts of the body due to gravity (Vass 2001). This causes discoloration of many body parts, ranging from bright red to dark purplish (Joshi 2021). When looking to find the PMI of natural mummies or any discovered body, the decomposition of the body and other indicators, such as insect activity and weather conditions, are considered when analysts do their estimation.

In most situations, human decomposition begins within minutes of death and can be calculated in simple formulas. In his research, Arpad Vass (2001) created the formula $y=1285/x$, calculating the number of days (y) it takes for a body to become fully skeletonized or mummified at a set temperature (x). For the example provided, a body in an ambient temperature of 10°C would take about 128.5 days to become skeletonized (Vass 2001). The breakdown of a body after death is caused by a process called autolysis, or self-digestion, and is then followed by putrefaction (Vass 2001). As the cells in the body are deprived of oxygen, carbon dioxide levels in the blood rise, pH levels decrease, and wastes begin to accumulate and poison the cells. Unchecked cellular enzymes, such as lipases, proteases, and amylases, start dissolving the cells from the inside out, causing them to rupture and release nutrient-rich fluids (Vass 2001).

This process occurs more rapidly in tissues that have a high enzyme and water content, like the liver and brain, but eventually affects all the cells in the body (Vass 2001). It takes a few days for autolysis to become visually apparent, first seen by the appearance of fluid-filled blisters on the skin and skin slippage, where large sheets of skin slough off the body (Byers and Rhine 2017).

Once cells have ruptured, the release of nutrient-rich fluids triggers the commencement of putrefaction, a process involving the breakdown of soft tissues by microorganisms such as bacteria, fungi, and protozoa (Hayman and Oxenham 2020). As the process continues, the breakdown of proteins leads to the release of sulfur-containing compounds such as cysteine and methionine. These sulfur-containing compounds react with the iron in hemoglobin, causing it to form sulfhemoglobin. This results in the catabolism of tissues into gasses, liquids, and simple molecules (Hayman et al. 2020). The first sign of putrefaction is a greenish discoloration of the skin which is caused by the formation of sulfhemoglobin. When hemoglobin is oxidized, the iron atoms are replaced by sulfur atoms, which gives sulfhemoglobin its greenish color (Hayman and Oxenham 2020). As the process continues, various gases accumulate in the bowels, distorting tissues and potentially causing skin rupture, which may then cause postmortem injuries (Vass 2001). It is important to note that the green color is not always present during putrefaction and can depend on various factors, such as the amount of hemoglobin in the blood, the pH of the tissues, and the presence of other chemicals in the body.

The final stages of decomposition include skeletonization, where soft tissue is removed, leaving only bones, and skeletal erosion, which is the loss of bone tissue due to various factors. Since these stages are determined by bone status rather than tissue status (Lennartz 2018), they are not the main focus of this paper.

The stages of decomposition to establish the PMI are a critical aspect of forensic investigations, and a few standardized methods have been developed. The Megyesi (2005) approach to decomposition scoring quantifies the decomposition process by identifying distinct stages and measuring the accumulated degree days (ADD). This approach is an expansion of the descriptive model, which only provides a general overview of the stages of decomposition (Lennartz 2018). ADD is the measure of the amount of heat energy required for decomposition to occur. This measure is based on the principle that the rate of decomposition is influenced by temperature. By calculating the accumulated degree days, researchers can estimate the time it takes for a specific stage of decomposition to occur. Despite the widespread use of Megyesi and colleagues' total body scoring method, the ADD model does not account for the state of mummification since the body is unlikely to progress to skeletonization, even as degree days continue to accumulate. This is because mummification is a state of halted decomposition, which does not lead to complete skeletal erosion (Lennartz 2018). Therefore, the ADD model may not be an accurate tool for estimating the PMI in mummification cases (Lennartz 2018).

Traditionally, determining PMI involves examining physical changes in the body, such as rigor mortis, lividity, and decomposition. However, natural mummies do not follow the standard decomposition path, making it more challenging to determine PMI using these methods. For example, the skin and organs may be well-preserved in some cases, while in others, they may be wholly desiccated (Gitto et al. 2015). When determining a PMI while analyzing a naturally formed mummy, researchers must use multiple methods. Along with traditional methods described above, new technologies in the field are being used to advance the field when trying to establish PMI from natural mummies.

Post-mortem Interval Technologies and Natural Mummies

Weight of Natural Mummies to Find Post-mortem Interval

In a study that reviewed the autopsies of 20 cases of forensic natural mummies, researchers investigated the different methods used to determine the PMI. One approach was to simply weigh the mummified body. This method was based on the premise that as a mummy ages, the skin and other organic materials that make up the body can become desiccated (Leccia, Alunni and Quatrehomme 2018). In this method, the entire mummified body is weighed, including any remaining organic tissues and bones. It is important to note that even in natural mummies, some organic material may still be present. One of the investigators looked at the weights of a mummy to try to establish PMI. Mummies were classified as either heavy, which would correlate with a PMI of several weeks to several months, to light, with a PMI range of several years, and finally, very light, which could be centuries old. (Leccia, Alunni and Quatrehomme 2018). Only two of the 20 mummies from the cases looked at were weighed at the time of their autopsy: Case #18, whose full body weight was 20 kg and had a PMI of three years, and Case #19, who weighed 29 kg with a PMI of four to five weeks (Leccia, Alunni and Quatrehomme 2018).

However, it is important to note that the weight of a mummy may not always be an accurate predictor of PMI, especially in cases where the preservation conditions were unique. The use of body weight as an indicator of PMI has both advantages and disadvantages: one advantage is that it is a simple and non-invasive method that investigators can use with minimal training. Additionally, it can provide a rough estimate of the PMI, which can be useful in initial investigations. However the weight of a mummy can be affected by various factors, such as the method of preservation, the environment, and the initial weight of the body before mummification. Therefore, the weight-based method of estimating PMI should be used in conjunction with other techniques to obtain a more accurate estimate. As well, the weight of mummies from a bog environment can vary greatly depending on both the degree of preservation and the amount of water they have absorbed over time, as compared to other natural mummies from dry environments that tend to have lower weights due to the dehydration process that occurs in these environments (Sivrev et al. 2004).

Stable Isotopes to Find Post-mortem Interval

Studying stable isotopes is important for forensic anthropologists as well as archaeologists in their PMI estimations because stable isotopes can be used as a natural clock to provide information about an individual's diet, geographic origin, and time of death (Bartelink and Chesson 2019). Stable isotope analysis of carbon and nitrogen in bone or hair samples can reveal information about an individual's diet, such as whether they were subsisted on C3 or C4 plants, or freshwater or marine resources. Additionally, stable isotope analysis of strontium and oxygen can provide information about an individual's geographic location, because there is variation in the isotopic composition of these elements across different regions. By comparing the isotopic signature of the individual's tissues to the known isotopic signatures of different regions, researchers can estimate the individual's place of origin. This information can also be used to establish a timeline for the PMI.

There are many investigations exploring the altering of stable nitrogen isotopes ($\delta^{15}N$) during decomposition as a means of determining PMI, with two critical principles to consider: comprehending the purpose of $\delta^{15}N$ values and understanding the changes that nitrogen undergoes during muscle tissue decomposition. Nitrogen has two steady isotopes, $\delta^{15}N$ and $\delta^{14}N$, which react differently in chemical reactions, leading to the preference of one isotope over the other (Hayman et al. 2020). Muscle tissue putrefaction causes the breakdown of amino acids; this breakdown is a chemical process that involves the breaking of the peptide bonds. These are the chemical bonds that form between the carboxyl group (COOH) of one amino acid and the amino group (NH_2) of another amino acid, creating a peptide linkage essential to hold the amino acid together (Hoffman and Rasmussen 2022). This process is catalyzed by enzymes produced by bacteria that colonize the tissue after death. The breakdown of amino acids generates a range of volatile compounds, such as ammonia (NH_3), putrescine ($NH_2(CH_2)4NH_2$), and cadaverine ($NH_2(CH_2)5NH_2$) in smaller quantities (Hayman and Oxenham 2020). As putrefaction proceeds, the chemical reactions responsible for generating these gases preferentially select the lighter isotope ($\delta^{14}N$), causing the heavier isotope ($\delta^{15}N$) to become more concentrated in the remaining muscle tissue. As a result, the concentration of $\delta^{15}N$ will increase over time relative to the concentration of $\delta^{15}N$ in fresh muscle tissue (Hayman et al. 2020).

A study by Keenan and DeBruyn (2019) looked at three types of nonhuman vertebrate samples to establish methods for determining PMI from stable isotope analysis. The researchers set up three sample groups and placed them in locations free from scavenging where they could naturally decompose. A primary sample group was collected from animals with a known PMI. The next sample group was collected through the means of road kill, which offered an unknown PMI. The last sample was collected through archived samples mainly provided by museum collections, with a mix of known and unknown PMI.

The researchers removed decomposing tissue at certain intervals and examined their carbon and nitrogen isotopic values (Keenan and DeBruyn 2019). They then placed their values into the δ notation for stable isotope analysis. The δ notation is a commonly used method to express the relative abundance of stable isotopes in a sample, typically compared to a known standard (Hoffman and Rasmussen 2022). It measures the difference in the ratio of the stable isotope to its more common counterpart (usually expressed as ppt or ‰) between the sample and the standard.

Once the isotope ratio was determined, Keenan and DeBruyn (2019) then used the Vienna Pee Dee Belemnite model as the standard for comparison in determining the carbon and oxygen isotopic composition in the experimental groups (Hoffman and Rasmussen 2022). The results of their study showed PMI could potentially be estimated from the increase of $\delta^{15}N$ on various vertebrate soft tissues. The researchers did state that further studies are necessary to validate the findings and assess potential limitations and sources of error (Keenan and DeBruyn 2019).

X-Rays and Computed Tomography to Find Post-mortem Interval

X-rays have been a valuable tool in the study of mummies and bog bodies for over a century and have helped determine the age of death and PMI of these individuals. X-ray and CT scans provide a non-invasive way to visualize the internal structures of these remains without causing damage to the delicate tissue (Cox 2015). X-rays have been used to search for amulets in Egyptian mummy wrappings and to determine the sex and age of the mummy based on skeletal traits

(Lynnerup 2009). One of the most significant uses of X-rays in forensic science is the production of 3D solid models of skulls for facial reconstruction. CT scanning has become the preferred method, but X-rays paved the way (Lynnerup 2009).

The first CT scanning of mummies occurred in 1977, marking a significant advancement in the non-invasive analysis of ancient remains. The first CT scan was of the brain in 1977 (Cox 2015); from there, whole-body scans were conducted on 11 mummies. The CT scans were considered the most comprehensive scanning of mummies up to that point. The images produced through this process revealed important information about the internal structures of the mummies, allowing researchers to study details that would have been impossible to access otherwise. Since then, the use of CT scanning in the analysis of mummies and other ancient remains has become increasingly popular. The use of non-invasive methods, such as CT scans, have also allowed for cost-cutting in forensic investigation, by allowing the medical examiner to determine whether a complete, partial, or external examination is necessary for a case. It is also beneficial to respect the wishes of a family who would prefer no autopsy (Adolph 2022).

The CT scanner takes individual X-ray image slices and reassembles them using different algorithms to connect the boundaries between them. While hospitals have access to CT scanners equipped with computer programs designed for medical purposes, it is important to note that these programs are tailored for living tissues and organs (Lynnerup 2009). Living bone typically has a consistent density, making it easy to pre-program a 3D rendering of skeletal tissues for a scanned patient.

Some studies have attempted to establish regression equations when trying to determine PMI using CT scan. By using spiral CT scans on adult rabbit models to observe changes in CT values and organ morphology at different PMIs (. The experimental group underwent whole-body CT scans at different PMIs, while the comparison group underwent traditional anatomy at each PMI. Organs of focus were the liver, heart, lungs and brain tissues. From these findings, they were able to build a binomial regression equation and the related coefficient (R2) for the CT values of different organs and PMI time points using SPSS (Wang et al. 2017). It is important to note that this study used rabbits and not human corpses. The rabbits were also held in locations that had controlled temperature and humidity, which does not reflect real-life scenarios.

Current methods for establishing PMI from CT scans can be more complex for mummies and bog bodies as extensive diagenetic changes to the remains poses unique challenges (Lynnerup 2009). Unlike cadavers, bog bodies have undergone such significant changes that the remaining tissues, including the bones, are severely degraded. The acidic environment of the bog can cause calcium to leach out of the bones, which leads to demineralization and a loss of bone hardness (Lynnerup 2009). Consequently, when X-rayed, bog body bones are often poorly visualized, appearing transparent like glass. This poses a challenge for applying the same Hounsfield unit (HU) range used clinically to CT scan bog bodies (Adolphi 2022). HUs are critical to CT imaging, providing a quantitative measure of the relative density of different body tissues based on a calibrated gray-level scale. For example, the air is assigned a value of -1000 HU; water is assigned 0 HU, and bone density is assigned +1000 HU (Silva et al. 2012). In medicine, HUs are useful for diagnosing and treating various diseases, and in forensics, they help to analyze and identify remains, including bog bodies and mummies. The demineralization is not uniform and may vary within the skeletal system or even within a single bone due to the diagenetic microenvironment, creating a patchy appearance of the bone, despite the bone being structurally intact (Lynnerup 2009).

Another notable difference between CT and X-rays is the change in the radiodensity of certain tissues (Lynnerup 2009). Unlike in clinical medical imaging, where tissues are categorized into four degrees: air, fat, soft tissue, and bone, the radiodensity of bog bodies may exhibit a different radiodensity structure due to the deposition of soil mineral salts containing metals like iron (Ahern and Brygel 2014). This deposition can produce more radiodensity in specific tissues such as ligaments, fasciae, and the subcutis. This change in radiodensity is likely due to the unique diagenetic environment in which bog bodies are preserved and underscores the importance of adapting medical imaging techniques to account for the specific properties of bog bodies (Lynnerup 2009).

DNA Sequencing to Find Post-mortem Interval

With the exception of red blood cells, DNA can be found in most cells in the human body. It is located in two distinct places in a cell: the nucleus and the mitochondria. The DNA in the nucleus is tightly packed into chromatin and is referred to as nuclear DNA (Gandolfo-Muller 2021). During DNA replication, the chromosomes unwind and are passed down from parent to child, thereby establishing the principle of heredity. Gene expression refers to the process by which the genetic information contained in a gene is used to produce a functional product, such as a protein or RNA molecule.

The process of gene expression begins with transcription, in which the DNA sequence of a gene is copied into a messenger RNA (mRNA) molecule. This mRNA molecule then moves out of the nucleus and into the cytoplasm, where it serves as a template for translation (Elmore 2007). After death, gene expression gradually declines as cells and tissues stop functioning and eventually break down. However, some genes may continue to be expressed at different rates and durations depending on a number of factors, including the type of tissue, and the cause and manner of death (Fulda et al. 2010). For example, some genes involved in inflammation, stress responses, and cellular repair may be upregulated shortly after death as the body responds to trauma and injury. Other genes, such as those involved in DNA repair and energy metabolism, may be downregulated as cells and tissues become deprived of oxygen and nutrients (Elmore 2007).

Gene expression analysis is a widely used tool among scientists to explore the underlying biological pathways and protein functions involved in various biological processes. Recently, forensic science has also recognized the potential of gene expression analysis in predicting an individual's death time (Iskandar 2020).

The expression of genes can vary greatly depending on an individual's physiological state, and other factors, such as disease, injury, and environmental stress, can influence this variation. After death, gene expression levels can change rapidly and consistently over time due to the degradation of cellular structures and metabolic processes (Zhu et al. 2017). Forensic researchers have discovered that by analyzing the changes in gene expression patterns in postmortem tissues, they can potentially determine the time of death with a greater degree of accuracy than traditional methods, such as rigor mortis or body temperature measurement. This approach, known as a molecular autopsy, involves identifying and analyzing specific genes known to change after death.

Conducted by the National Institute of Health, the Genotype-Tissue Expression

(GTEx) Project looked into the use of gene expression to determine a PMI (Zhu et al. 2017). 50 types of human tissues were collected from hundreds of human donors with a goal of building a comprehensive and repeatable model for determining PMI from mRNA degradation. From their samples they sequenced entire genomes, isolated nucleic acids, and recorded postmortem changes over 27 hours. To isolate the genes believed to be associated with PMI, they used multiple linear regression models. To reduce false positive results in the tissues they tested, researchers would repeat the test 10,000 times (Zhu et al. 2017). Their results showed that 7,546 genes were associated with postmortem up- or down-regulation in 15 human tissues tested (Zhu et al. 2017). These results offer crucial insights for researchers analyzing gene expression patterns using postmortem tissue samples.

An experiment done by Marissa Gandolfo-Muller (2021) at the University of Southern Mississippi examined the amount of forms of DNA degradation of natural mummies in a variety of environments. Mummies were created to match known mummification environments, such as open air environments, closed air, buried in soil, desert, rock salts, caves, environments of freezing temperatures, saline lakes, and bog bodies. Using a terrarium with peat moss, distilled water, lactic acid powder, and liquid humic acid, they simulated a bog environment. Specimens were placed in the terrarium for 10 weeks, and their DNA was then extracted for analysis. The study showed excellent preservation of soft tissue and successful DNA extraction and amplification (Gandolfo-Muller 2021).The DNA from the bog body showed more preservation than most samples of mummies that were persevered in dryer processes but lower preservation in others. More studies should be conducted to see the effects of the acidic environment on DNA degradation to help advance the method of PMI estimates from DNA.

Proteomics to Find Post-mortem Interval

Proteomics is a new field of study involving molecule change of proteins (Procopio et al. 2018). There have been many promising advances in the area. A proteomic study conducted at Chungnam National University built a profile for PMI using skeletal muscle tissue from rat and mouse models and samples collected from three autopsies (Choi et al. 2019). They prepared each sample at a controlled location so they could monitor the degradation of novel proteins. Conducting SDS-PAGE and Western blot experiments, they demonstrated that two proteins could serve as prospective indicators of postmortem changes in skeletal muscle. GAPDH, an enzyme that regulates mRNA stability, and eEF1A2, a transporting protein that attaches aminoacyl-tRNA to the A-site of the ribosome, were the novel proteins that followed a consistent pattern during decomposition (Stelzer et al. 2016). Their results showed that the analysis of postmortem GAPDH resulted in distinct single bands at approximately 40 kilodalton (Kda) that decreased at around 24 hours (Choi et al. 2019). This continued until hour 96, when it reached a significance level of $p = 0.016$. The analysis of eEF1A2 during decomposition also showed a 5% decrease in the rat and mouse models. The animal models displayed a comparable pattern of protein degradation when compared to the human samples. This study helps to confirm the potential significance in future forensic proteomics and bolsters the use of animal models in fundamental studies on estimating PMI.

Studies conducted using bone proteomic analyses in simulated forensic scenarios focus on identifying specific biomarkers that could aid in estimating the PMI. In one study using pigs as animal models, findings indicated that a decline occurred in certain plasma and muscle proteins as PMI increased and an elevation occurred in the deamidation of biglycan, a protein involved in bone growth and mineralization (Procopio et al. 2018).By looking at multiple proteins over prolonged PMI, it was recorded that most of the proteome changes occurred over the first four months (Procopio et al. 2018), with slower decay in the proteins thereafter.

Proteomic studies have also been done on one of the world's most well-studied natural mummies, the Tyrolean Iceman from the Copper Age. The Tyrolean Iceman is considered a wet mummy, yet it is preserved differently from the wet mummies formed in bog environments (Maixner et al. 2013). It was not the make up of his microbial environment that helped to preserve him but the “humidity [that] was retained in his cells while he was naturally mummified by freeze-drying” (Maixner et al. 2013). For over 5300 years, he has been preserved in time and now, with modern technologies, his proteins can be sequenced alongside his DNA. The two samples of brain tissue from the

Tyrolean Iceman was the first attempt at a paleoproteomic research (Maixner et al. 2013). Though this study did not focus on establishing a PMI, it did give fantastic insight into the possibilities of what proteomics can offer. So far 502 unique proteins have been identified from the Tyrolean Iceman samples.

Conclusion

Natural mummies, such as bog bodies, provide valuable insights into the past by preserving organic material that would normally decompose. However, this unique preservation process also poses significant challenges for researchers trying to estimate the PMI. Traditional methods for estimating PMI rely on the rate of decomposition and other physical changes that occur in the body after death. However, these methods may not be reliable for bog bodies because decomposition is often interrupted or altered by the bog's acidic environment, making it difficult to determine how long the body has been preserved. Furthermore, there is a lack of standardization in methods and protocols for estimating PMI, which can further complicate the process for forensic anthropologists, pathologists, and archaeologists.

Despite these challenges, recent advances in scientific methods, such as stable isotope analysis, DNA sequencing, and proteomics, have shown promise in improving the accuracy of PMI estimations. Stable isotope analysis can also provide insights into the diet and geographic origin of the individual. DNA sequencing and proteomics can provide information about the person's age, sex, and health status, which can also be used to estimate the PMI. By combining these newer methods with more traditional methods, researchers may be able to improve the accuracy of PMI estimations for natural mummies. Overall, thanks to these advances, researchers will be better able to understand the unique processes involved in the formation of natural mummies, such as bog bodies, and be able to adjust their methods for estimating PMI. This knowledge will ensure that the valuable insights provided by natural mummies are not lost.

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