Chapter 14
Decomposition of Human Remains

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Introduction

Early scientific research into "putrefaction" by eighteenth century physicians was driven by a need to understand and treat living patients who were suffering from "putrid diseases" (presumably conditions such as treponemal disease, non-specific osteomyelitus, bacterial skin infections, abscesses, and the like, which could result in the formation of necrotic tissue, but which today can be treated by modern medicine).[1,2] But these works clearly recognized and tried to seek explanation to some of the fundamental microbially induced changes in the human body, in particular, to soft tissue that occur during different stages in the decomposition process and which result in pH change, and the evolution of volatile compounds. As such, these works are an early precursor to the discipline that today we know as "taphonomy". This term, originally coined by the Russian palaeontologist Ivan Efremov to describe the "transformations from the biosphere to the lithosphere"[3] in explaining the formation of fossils, today has much broader meaning. The term has been widely adopted in archaeology and forensic science and is concerned with the decomposition of the body and associated death scene materials. As such, the disciplines of archaeological taphonomy/diagenesis[4–7] and forensic taphonomy[8–11] cover the location of buried or disturbed human remains[12] and time since death/burial estimation, and explain the survival/differential decomposition of physical remains and macromolecules such as proteins, lipids, and DNA.

Death may be defined under two categories: somatic and cellular death.[13] In somatic death, while the person has lost sentient personality, reflex nervous activity often persists. In cellular death, the cells of the body no longer function, cease to exhibit metabolic activity, and cannot function by means of aerobic respiration.[13] Understanding the distinction between somatic and cellular death is important when considering physiological changes that occur immediately after death, when, for

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instance, the corpse still exhibits muscular contractions, but has less relevance to much more destructive, longer term decomposition.

After the cessation of heart function, the body becomes flaccid and blood ceases to circulate. The body goes through some well-documented changes, known as the "classic triad" of livor, rigor and algor mortis. That is, the blood drains to the lower areas, and the body stiffens and cools until it approaches ambient temperature. The rate at which these changes occur is largely governed by environmental conditions, especially temperature together with microbial load and diversity.

Post Mortem Hypostasis (Lividity, Livor Mortis)

One of the earliest effects of the heart ceasing to function is that blood will drain to the lower parts of the body under the influence of gravity, and this causes a characteristic discolouration of the dependant areas termed *post mortem hypostasis*. Depletion of oxygen from the blood results in a colour change from bright red to deep purple. The collection of this deep colouring in the lower parts of the body is apparent 1–2 h after death and becomes fully developed within about 6 h and firmly fixed after 12 h.

Rigor Mortis

Except in the cases that exhibit cadaveric spasm, the first effect of death in most cases is a general relaxation of muscular tone. The lower jaw drops, the eyelids lose their tension, the muscles are soft and flabby, and the joints are flexible. Within a few hours after death, and generally while the body is cooling, the muscles of the eyelids and the jaw begin to stiffen and contract followed by similar changes in the muscles of the trunk and limbs so that the whole body becomes rigid.[13] The muscular tissue passes through three phases after death:

1. It is flaccid but contractile, still possessing cellular life
2. It becomes rigid and incapable of contraction, being dead and
3. It once more relaxes but never regaining its power of contractility

Rigor mortis is caused by the breakdown of adenosine triphosphate (ATP) and the build-up of lactic acid to about 0.3% in the muscle tissues. At this point the muscles go into an irreversible state of contraction. In temperate climates this condition usually commences within 2–4 h of death, reaching a peak at 12 h, and starts to disappear at 24 h, with the cadaver becoming limp within 36 h. The flaccidity that follows stiffening is due to the action of alkaline liquids produced by putrefaction. In contrast to rigor mortis, cadaveric spasm is a rare phenomenon of the instantaneous stiffening of specific muscle groups occurring at time of death, e.g. the hand clutching a weapon. It is usually associated with sudden, violent death.[14]

Cooling (Algor Mortis)

After death the body starts to cool, because of loss of living body heat to the external environment. The rectal temperature of a healthy adult at rest is approximately 99°F with daily variations up to 1–1.5°F. The temperature varies throughout the day, being at its lowest between 2 A.M. and 6 A.M. and highest between 4 P.M. and 6 P.M. The rate of cooling is determined by the difference in temperature between the body and its environment. For instance, in temperate climates it has been suggested that for an average adult the heat loss in air will be 1.5°F/h, while in tropical climates it is 0.75°F/h.[14,15]

Under most environmental conditions body decomposition will eventually result in the loss of soft tissue, leaving the skeletal elements. However, the study of archaeological bone has indicated that there may be residual organic material, e.g., bone collagen, surviving even after hundreds of years of burial.[16,17] In addition, there has been considerable interest in the survival of DNA from heavily degraded remains both from the point of victim identification during the investigation of mass graves, natural disasters,[18] and the recovery of ancient DNA in archaeological studies.[19,20] In addition, research has been ongoing to document the body decomposition products that are left in transit graves, where a body has been temporarily buried, or on a surface where a body has lain for time since death estimation.[21] Thus, a detailed understanding of not only the gross loss of soft tissue but also the chemistry of surviving organic molecules in bone and the soil is of importance to both forensic and archaeological scientists.

As a body decomposes, soft tissues will progressively liquefy. There are a number of processes that cause this: the body's own enzymes will self-digest material at a cellular level in a process known as *autolysis*, while the usually much more destructive process of *putrefaction* is driven by bacterial enzymes. The bulk of these putrefactive microorganisms are anaerobic and are derived from the body's own gastrointestinal (GI) tract, and their activity during the major phase of tissue breakdown keeps the tissue mass anoxic. At later stages of decomposition, extracorporeal microorganisms such as soil fungi[22] may be involved, but these can only be associated with the exterior of the body mass or after the major phase of decomposition when the remaining material is better oxygenated.

Despite the possible actions of insects or scavenging animals, it is microorganisms that consistently play a fundamental role in the decomposition of human remains. While there are recognizable changes that a body may proceed through (e.g., from putrefactive decomposition towards skeletalization), there are key variables that influence the advancement or retardation of this process. Of greatest note are the environmental constraints of temperature, moisture content, and their influence on tissue. At one extreme is the process of desiccation (natural mummification) that can retard decomposition because of the drying of tissue below a critical threshold for bacterial action. This, of course, does not preclude superficial mould growth on the outside of partially desiccated remains.

Cadaveric Decay

Decay of the body is dominated by the two destructive processes of autolysis and putrefaction. Autolysis occurs independently from any bacterial action, while putrefaction, the reduction and liquefaction of tissue, is a microbiologically dominated process.

Autolysis is a process of postmortem self destruction due to intrinsic enzymes at a cellular level. It is not apparent at a macroscopic level but can be documented histologically. Importantly, it operates without the participation of bacteria.[23–25] The postmortem release of intra- and extracellular hydrolytic enzymes denature molecules and cell membranes. Cells become detached and the cell contents are broken down.[23] The partial destruction of cellular structures will greatly facilitate further bacterially driven putrefactive change. The breakdown results from the action of bacteria and enzymes that are already present in the tissues, or enzymes, which are otherwise derived from soil microorganisms and fungi.[26,27]

The tissues of a corpse are considered to be free of microorganisms within the first 24 h following death. It is likely that a lot of the microorganisms that are circulating through out the body are continually being deactivated, as the immune responses of the body are still active over 48 h after death. After death, the microorganisms present in the body (e.g., in the GI tract) initially invade the local tissues and gain access to the rest of the body (including bone which has a good blood supply) via the vascular and lymphatic systems. As the redox potential of tissues is known to fall very quickly flowing death, the growth of obligate aerobes is substantially reduced so that bacteria such as Micrococci, *Pseudomonas* and *Acinetobacter* spp. are the only remaining viable bacteria that are found at the outer surfaces of the decaying tissue. Anaerobic bacteria become generally more prevalent.[28] While the human GI tract is composed of a very complex microbiology, only a small number of bacteria, i.e. *Clostridium* spp., Streptococci, and the Enterobacteria during the first days of death, are involved in the putrefaction process.

Putrefactive change is usually first visible on the abdominal wall, owing to the conversion of haemoglobin by anaerobic bacteria. Initial activity is usually documented in the region of the right iliac fossa where the caecum is relatively superficial.[13] Putrefaction results in widespread decomposition of the body caused largely by action of bacterial enzymes, mostly anaerobic organisms from the bowel. The process of putrefaction commences immediately after death and is visible under normal conditions from 48 to 72 h afterwards.[27] Initial signs of putrefaction are green or greenish-red discolouration of the skin of the anterior abdominal wall due to the formation of sulph-haemoglobin. This spreads to the whole of the abdominal wall, chest, and thighs, and eventually to the skin of the whole body (marbling). This usually takes about 7 days. Over time, the corpse’s skin begins to go greenish owing to the formation of sulph-haemoglobin in settled blood. The gases that are generated during this decomposition process include hydrogen sulphide, carbon dioxide, methane, ammonia, sulphur dioxide, and hydrogen. These gases increase to high levels in the large bowel and around tissues that are being broken down by natural autolysis and bacterial lysis.[29] Further anaerobic fermentation in the corpse results in the development of more by-products, specifically volatile fatty acids. Over time, the natural process of putrefaction of hydrocarbons, ammonia compounds, and biogenic amines begin to accumulate in the corpse.

Because of the changes that begin to occur in the body, the indigenous microbiota that still exists, particularly the GI flora, increase their proliferation, accelerating the whole decomposition process. It has been documented that 90% of microorganisms isolated from tissue from human corpses are strict anaerobes. The highest concentrations of bacteria isolated include mainly Gram-positive non-sporulating anaerobes such as bifidobacteria. Lower numbers of Lactobacillus, *Streptococcus* spp., and bacteria belonging to the Enterobacteriaceae group have been observed. Other bacteria that have been isolated from decomposing tissues, but in lower numbers than the bacteria mentioned above, have included *Bacillus* sp., yeasts, *Staphylococcus* spp., and *Pseudomonas* sp.[30,31]

In the early stages of decomposition, bacteria isolated from human corpses have included, among others, *Staphylococcus* sp., *Candida* sp., *Malasseria* sp., *Bacillus* sp., and *Streptococcus* spp. While a number of non-fastidious bacteria have been identified from decaying human matter, because of the very high abundance of microorganisms associated with the host a major overgrowth of bacteria both culturable and non-culturable would be inevitable because of the availability of a food source. These include, among others, micrococci, coliforms, diptheroids, and *Clostridium* spp. Also, organisms such as *Serratia* spp., *Klebsiella* spp., *Proteus* spp., *Salmonella* spp., and bacteria such as Cytophaga and Pseudomonads and flavobacteria have been documented to be evident. Also, the host's "normal" microbiota will become mixed with environmental microorganisms such as Agrobacterium, amoeba, and many fungi, which are also significant to human decomposition.

From a microbiological point of view it is plausible to suggest that every microorganism, both endogenous and exogenous, of the host is involved in some aspect of the human decomposition process. Ultimately, the decomposition of human remains would not progress without these normal microbiota and external exogenous microorganisms developing a community in the form of different biofilms. The formation of the biofilm will enhance the continual survival of the host microbiology, but this time the community will become more detrimental to the host rather than beneficial. As has been outlined, the role of microbiology in human decomposition is significant. This role is more apparent when we consider bodies that have open wounds (e.g., death due to stabbing), as these undergo faster decomposition than bodies without wounds. This is principally due to the prevalence of high levels of bacteria within the wounds.[30] In addition to this, if a person dies as a result of bacterial or viral infection, postmortem alterations are accelerated.[32]

Intrinsic Microorganisms and the Chemistry of Death

The human body is composed of approximately 64% water, 20% protein, 10% fat, 1% carbohydrate, and 5% minerals.[33] Adipose tissue is on average 5–30% water, 2–3% protein, and 60–85% lipids, of which 90–99% are triglycerides,[34] while muscle largely consists of protein. Soft tissue decomposition is characterized by the progressive breakdown of these proteins, carbohydrates, and fats. The soft tissues eventually liquefy and disintegrate, leaving skeletalized remains articulated by ligaments.[24,27,35]

Protein Decomposition (Proteolysis)

Protein is broken down by enzyme action, but this does not proceed at a uniform rate throughout the body. The rate is determined by the amount of moisture, bacterial action, and temperature. Moisture favours decay, and proteolysis is slowed by cooling and increased by warming.

Soft tissue proteins such as those forming neuronal and epithelial tissues are destroyed first during decomposition, i.e., the lining membranes of the GI tract and pancreatic epithelium.[25] At an early stage of decomposition, proteins forming the brain, liver, and kidneys are also subject to putrefactive change, while proteins such as epidermis reticulin and muscle proteins are more resistant to breakdown.[23] The most resistant proteins are those associated with connective tissue and cartilage.

Within the hard tissues, proteins such as type I collagen (comprising 90–95% bone protein, alongside other proteins such as osteocalcin, osteopontin, and osteonectin) and amelogenin within tooth enamel are protected by their association with biological apatite. While these are subject to biological or chemical attack under many conditions, they exhibit resilience and as such persist into the archaeological record. These biomolecules have been the subject of considerable interest and utility within the archaeological science community.[36–38]

Keratin, which is an insoluble protein found in hair, nail, and skin can only be exploited as a nutrient source in the first instance by specialized keratinolytic microorganisms.[39,40] Given that the hair shaft is a complex heterogeneous structure, it is hardly surprising that microbially induced changes occur selectively on the basis of the relative resistance of these morphological structures to chemical enzymatic attack.[41] Where the depositional conditions are favourable, hair and nail can persist over considerable timescales, on naturally mummified and even on otherwise skeletal remains.[42]

Common bacteria that are very proteolytic and therefore are involved in protein breakdown include *Pseudomonas*, *Bacillus*, and *Micrococcus* spp. As well as these bacteria, sulphate-reducing bacteria found in the GI tract have a vast array of enzymes and have the ability to utilize sulphates and sulphur-containing compounds and as such are important bacteria in human decomposition.

In general, proteins break down into peptones, polypeptides, and amino acids, a process known as *proteolysis*. Proteolysis leads to the production of phenolic substances, and gases such as carbon dioxide, hydrogen sulphide, ammonia, and methane. The sulphur-containing amino acids of the proteins such as cysteine, cystine, and methionine undergo desulfhydralation and decomposition by bacteria, yielding hydrogen sulphide gas, sulphides, ammonia, thiols, and pyruvic acid. Thiols or mercaptans are decomposition gases containing the –SH (sulfhydryl group), and these are responsible for the very bad odours generated during human decomposition.

Protein decomposition also results in the production of a range of organic acids and other substances that become bacterial metabolites. These are generally of low or moderate molecular weight, anionic or non-ionic, and are susceptible to rapid breakdown by bacteria.[43]

Decomposition of Fat

Human body-derived lipids comprise 90–99% triglycerides, which contain numerous fatty acids attached to the glycerol molecule. The body's adipose tissue consists of approximately 60–85% lipids, with most of the remainder being water.[25] Of those fatty acids making up the composition of adipose tissue, mono-unsaturated $C_{18:1}$ oleic acid is the most widespread, followed then by polyunsaturated $C_{18:2}$ linoleic acid and monounsaturated $C_{16:1}$ palmitoleic acid and the corresponding saturated $C_{16:0}$ palmitic acid.[25]

For the most part, hydrolysis rather than oxidation dominates the fat degradation system, largely because of the fact that bacterial action will have driven the tissues into an anaerobic condition. Palmitic acid increases and the oleic acid becomes increasingly reduced in amount through hydrolyzation. Neutral fat undergoes hydrolysis during decomposition, resulting in the formation of fatty acids hydrolyzed by lipases. This proceeds slowly and the activity of this enzyme system soon diminishes. Analyses of postmortem fat exhibit the presence of oleic, palmitic, and stearic acids as soon as 8 h after death. These are the first phase of neutral fat breakdown. Neutral fats that have been hydrolyzed produce a large concentration of unsaturated fatty acids resulting in the production of aldehydes and ketones. Hydrolysis of triglycerides results in the formation of glycerine and free fatty acids. Bacterial enzymes lead to the transformation of unsaturated into saturated fatty acids. Fatty acids, the products of hydrolysis, will be quickly oxidized into aldehydes and ketones; this, however, can only take place in the presence of oxygen.

More effective than the intrinsic lipases are the lipolytic enzymes produced by bacteria, particularly those of Clostridia (especially *Cl. perfringens*), which derive from the GI tract[44] and are able to grow at relatively high redox potentials.[45] These lipolytic enzymes significantly aid the anaerobic hydrolysis and hydrogenation of fat under warm conditions.[27] Water is necessary for both the intrinsic and bacterial enzymes to work, though there is usually sufficient moisture in the fat tissue itself. If the process continues, the neutral fat is totally converted to hydroxy fatty acids, which are deposited in its place.[46] If no further chemical changes take place, these fatty acids remain as adipocere (*adipo* = fat, *cere* = wax).

If the burial circumstances keep the oxygen levels low, then the fat degradation products will remain as adipocere.[47] Adipocere is a waxy substance that sometimes forms from the adipose tissue of dead bodies and has generally been considered to result from bacterial action, commonly in warm, damp, anaerobic environments.[48] The presence of bacteria and water is crucial for adipocere to form.[49] Adipocere is formed by the alteration of the soft tissue of the corpse into a greyish-white, soft, cream-like substance, over time becoming a solid and resistant compound. Adipocere is a soft, greasy material which may be white or stained reddish brown when recent. When adipocere is analyzed, in addition to stearic, palmitic, and oleic acids, there is a fraction of calcium soaps.[27] Old adipocere is white or grey, and depending on its age and condition it has been likened to suet or cheese.[27]

Extensive adipocere formation will be found on a body when conditions will allow only partial degeneration of fatty tissue, i.e., by hydrolysis and hydrogenation but not oxidative reactions. Adipocere in corpses has been found after as little as 30–90 days following death.[15]

Varieties of aerobic or facultatively anaerobic microorganisms from the surface of the adipocere have been identified. In culture, a number of Gram-positive bacteria, associated with the indigenous human microbiota, are able to degrade the adipocere. The role of bacteria in adipocere formation and degradation must be understood before we can use the presence of adipocere to extrapolate information about the post-death interval.[50]

Apart from corpse-specific characteristics (e.g., sex, age, physique, cause of death), method of burial (e.g., material of the coffin, depth of grave, individual or mass grave, clothing) and time of burial, the conditions of the resting place (geology, topography, soil properties and frequency of use, air, water, and heat budget) can have a special impact on adipocere formation.[51] It is a traditional belief that adipocere forms in damp environments – such as after submersion or interment in damp or waterlogged ground. Adipocere will also form in bodies buried in dry vaults, and in some cases distal elements (e.g., limbs and hands) have shown signs of mummification while adipocere is present in others. It is suggested that coffins will retard the rate at which adipocere forms but clothing enhances its formation.[52] Gas-chromatography-mass spectrometry was used to characterize the fatty acids from soils and associated tissues excavated from a 1967 Foot and Mouth burial pit. Subcutaneous fats were mainly composed of 55–75% palmitic acid, 17–22% stearic acid, and 3–16% oleic acid as well as 5–7% myristic acid. The distribution of fatty acids confirmed that the tissues had decayed to adipocere.[53]

There is little known about which specific microorganisms bring about lipid breakdown in soil, although it has been suggested that Gram-positive bacteria such as *Bacillus* spp., *Cellulomonas* spp., and *Nocardia* spp. are involved in the decomposition of adipocere.[50] In Brazilian cemetery studies, the presence of significant numbers but unspecified types of lipolytic bacteria (possibly *Clostridia* spp.) was reported for the groundwaters examined[54]; these were said to be directly related to the decomposition of the interred remains. Hydrogenation of fats under the influence of bacterial enzymes results in the partial conversion of unsaturated fatty acids into saturated fatty acids. As the fatty acids clearly have a bactericidal effect, further bacterial decomposition is stopped at this early adipocere stage. Additional microorganisms from outside can no longer penetrate when this hermetic seal is in place.[55]

Within the archaeological record, lipids are considered to be robust molecules and have been recovered extensively from human remains, soils, and in association with artefactual material such as ceramics.[56,57]

Decomposition of Carbohydrates

The utilization of carbohydrates present in the soft tissue of corpses occurs in the early stages of decomposition.[26] For example glycogen, a complex polysaccharide, will break down into sugars (glucose) by the action of microorganisms. Most sugars are completely oxidized to carbon dioxide and water, while some are incompletely decomposed. For example, *Clostridium* spp. breakdown carbohydrates to form a number of organic acids and alcohols, and fungi decompose the sugars to form organic acids including glucuronic acid, citric acid, and oxalic acid.[58] Postmortem production of alcohol by anaerobic fermentation occurs during conversion of body sugar to ethanol by bacteria, and this can begin within 6–12 h under hot, humid conditions.[10] In the presence of oxygen, the glucose monomer is broken down through the pyruvic acid, lactic acid, and acetaldehyde stages to form carbon dioxide and water.[58] Other gases produced through bacterial carbohydrate fermentation include methane, hydrogen, and hydrogen sulphide.

Decomposition of Bone

The loss of soft tissue from the corpse is referred to as *skeletonization*. The rate of skeletonization will depend on many factors such as whether the body is buried or not, depth of burial, temperature, moisture, and access by insects and larger scavengers.[30] Under anaerobic burial conditions or where the tissues have significantly desiccated, the rate of skeletonization will be low.[29] In these circumstances, the degree of soft tissue survival is often scored according to the region of the body, and survival of organs using indices such as the Aufderheide's soft tissue index.[42]

In addition to the loss of tissue from bone, the bone itself is subject to decompositional change.[29,59] Bone is a composite tissue having three main components: a protein fraction, collagen acting as a supportive scaffold; a mineral component, biological apatite to stiffen the protein structure; and a ground substance of other organic compounds such as mucopolysaccharides and glycoproteins.[60,61] Bone collagen and biological apatite are strongly held together by protein–mineral bonds, which give bone its strength and contribute to its preservation.[62]

The loss of protein and/or partial loss of bone mineral result in weakening/embrittlement, which is associated with bone buried over archaeological timescales when compared to fresh bone. The physical condition of excavated bone will depend on the integrity of the bone mineral bond due to collagen survival and the depositional environment. It is usual for archaeological bone recovered from aerobic, non-acidic environments to be stained but otherwise appear to be in good condition. However, cracking and flaking may occur on drying. In coarser, calcareous sand or loam where it is damp and more oxygenated, the bone surface will be rougher and may warp, crack, or laminate on drying, while material from coarse calcareous gravels will lose much collagen and have the consistency of powdery chalk and be coated in a white encrustation of insoluble salts. Bone from acidic peat deposits appears as interwoven fibres, is pliable, and hardens on drying.[63] Acid in soil is the most common agent of bone destruction and works by dissolving the inorganic matrix of hydroxyapatite which produces an organic material susceptible to leaching by water. The collagen fibres are sometimes preserved by natural tannins.[63]

Bone collagen is attacked by bacterial collagenases that hydrolyse the proteins to peptides; these are then broken down to form amino acids.[64] It has been suggested that collagen degradation is affected by the activity of the gas-gangrene bacterium *Clostridium histolyctium* which operates in a pH range from 7 to 8.[65,66] Alternative claims implicate bacterial collagenases as largely responsible for degrading bone collagen by reducing them to peptides that leach away in groundwater.[67] Regardless of the mechanism, once the protein mineral bond has been broken, the bone mineral is vulnerable to partial dissolution via chemical weathering.[68,69] Bones are generally better preserved in soils with a neutral or slightly alkaline pH than in acidic soils, which will result in the dissolution of biological apatite. Over short timescales, dry sand is an aid to preservation (although sandy soils are often acidic), as it retards bacterial decomposition, while in fine-grained soil and dense clay aerobic bacteria cannot live.

In archaeology there has been a lot of recent attention concerning the degradation of bone in the soil (diagenesis). The impetus for this work has been both an attempt to explain the differential survival of different elements of the skeleton as well as differential preservation between individual burials and to underpin more detailed biochemical analysis of archaeological bone based on the survival of organic matter such as bone collagen and DNA in teeth and bone.[19,20,37]

Extrinsic Organisms Involved in Human Decomposition

Colonization of the corpse by extrinsic organisms may begin within hours of death. Of particular significance are insects that are used as important forensic indicators to calculate time-since-death estimations and are discussed in greater detail elsewhere.[70–72] The attractiveness of a cadaver will depend on odour and fly oviposition, defined by temperature. Blow flies (Calliphoridae) may be attracted to the body within minutes of exposure, and gravid female blowflies will detect the presence of a body on the basis of a scent plume from some considerable distance. In laboratory experiments with caged *Callophora vicina* presented with baits that were fresh and partly decomposed, the flies ignored the fresh bait. Eggs are laid in natural body openings (mouth, nose, eyes, ears, anus, and open wounds) and up to 180 eggs can be laid at one time. Eggs take 1–2 days to hatch depending on temperature and humidity (low temperature retards insect activity, and 4°C or below is the lower threshold for hatching in *Callophora vicina*). The formation of maggot masses by these hatched larvae can cause massive soft tissue damage that will open tissue up to further putrefactive decay.

Burial will often pose a barrier to blow flies – although, if there is an opportunity for eggs to be laid prior to burial, then they can subsequently hatch and the larvae will feed. Sealed post-medieval coffins have yielded evidence of blowfly activity (pupal cases), which suggests that eggs were laid prior to closure of the coffin.[73] Over longer timescales, different insects will colonize the corpse. Although the desiccation of mummified tissue will inhibit normal putrefactive changes, they remain susceptible to insect attack by the larvae of the brown house moth or beetles such as *Dermestes lardarius* or *Necrobia rufipes*.

Larger animals such as domestic cats and dogs as well as foxes, badgers, and rats will scavenge meat from corpses. Whether they scavenge or not will be determined by ease of access as well as individual feeding preferences and the abundance of available food. Larger animals will dig up and disinter parts of corpses from shallow burials, and scatter skeletal elements from both buried and surface-deposited remains.[74,75] Importantly, in the case of buried remains, during the early putrefactive stages of decomposition such digging will disturb the grave and the resultant aeration of the grave will accelerate putrefactive change. This has been documented in experimental studies using pig burials as human body analogues.[76] The action of these larger scavengers is outside the scope of this chapter.

During the later stages of decomposition, microorganisms may be derived from the soil; here soil history is an important factor and will influence the size and nature of the population of dormant microorganisms. At the microscopic level, biological agents of decay include bacteria and fungi which can mimic pathological changes in bone.[77] Microscopic focal destruction of bone (tunnels) was first noted during the last century[78] and it is now understood to be caused by invading soil microorganisms,[79] possibly an unidentified mycelium-forming fungus.[80] Beginning at the surface of the cortex, the organisms proceed along the vascular channels and osteons, creating tunnels that expand until only separated by thin bars of hypermineralized bone. In the burial environment, it is believed that soil water content and temperature are important factors in focal destruction, which does not occur in wet, water-logged, or dry soils but is favoured in soils with moderate moisture in summer weather. Histological and physical (mercury intrusion porosimetry) analyses of bone from 41 archaeological sites across five countries revealed that the majority (68%) had suffered microbial attack.[81]

Most fungi that are found on decomposing remains are aerobic, and consequently their growth is restricted to the surface of the cadaver and little deep penetration of the tissues takes place. Fungi are commonly found on the skin and exposed surfaces of decomposing remains. In some cases they can also be found growing in the intestines and other body cavities. In addition, fungi may also be found growing in soil that is infused with decomposition products from the body.[82] Microorganisms may have complex inter-relationships and many synthesize antibiotic compounds. For example, griseofulvin is an anti-mycotic agent produced by *Penicillium griseofulvum*, and luteoskyrin is an anti-bacterial agent produced by *Penicillium islandicum*.[10]

If there is substantial surface vegetation, bone is susceptible to plant root damage, although this will be strongly influenced by seasonal effects. Physical damage by plant roots can mark, warp, and even break bones but the exact mechanism of biochemical plant root damage is obscure.[83] It is probable that plant roots manufacture mucilaginous substances that promote the growth of microorganisms when secreted. Certain microorganisms, including fungi, will discharge enzymes into the soil which catalyses the reaction that dissolves hydroxyapatite in bone and facilitates its absorption into the plant root system. A low pH will promote these chemical changes. Plants and their root systems constitute complex physical and chemical processes that efficiently breakdown the external and internal structure of human bone.[84] While it is appreciated that plant activity in the tropics is at a greater level than in the UK, the principle of vegetation acting as a decomposition vector of human skeletal remains is valid.

Environmental Controls that Promote and Inhibit Putrefactive Decay

There are a large number of inter-related factors that will affect the rate and nature of cadaveric decay. These principally include the condition of the body at the time of burial and the nature and circumstances of the burial environment.[85] A comprehensive survey of the issues has been reviewed by Mant,[85–87] which is a synthesis of results from over 150 exhumations carried out in Germany after the Second World War. These burials were made under a range of different circumstances and burial conditions: for instance, burial was often immediately after death rather than allowing for the normal postmortem interval of several days. In most cases, the dates of death and interment were known. It was not possible to carry out laboratory analyses of the tissues recovered, and the results are based on gross changes. From this data it is possible to examine the influence of a variety of factors on the decomposition of buried human remains. Some generalization can be made, although attention must be made to individual circumstances.[14] Thin bodies will skeletalize more rapidly than more fleshy ones in the same conditions. Antemortem or postmortem wounding makes cadavers more susceptible to invasion by extra-corporeal organisms than bodies that are buried with the skin intact, and will have a more rapid rate of skeletalization.[85]

The rate of decomposition of a body on the ground surface is more rapid than that of a buried body. This is due to the soil limiting the access by extra-corporeal microorganisms and larger animals as well as reducing the rate of gaseous diffusion. An oxygenated environment will increase the rate of human decomposition. Exposure above ground, even for a short period, will allow insects and carnivorous larvae to colonize the body and rapidly attack the soft tissue, a process which will continue after burial. Ambient temperatures above the ground are higher, there is more oxygen and less carbon dioxide, and access to the body is easy for scavenging mammals.

During initial cadaver decomposition, when the soft tissues lose their morphological structure, aspects of the burial environment that affect soil biology such as oxygen availability predominate and localized soil chemistry may be modified and dominated by the biochemistry of soft tissue decomposition. When the bulk of soft tissue decay has ended, then generalized soil chemistry may have a greater direct effect, for instance, on the corrosion of associated metals or in bone diagenesis. The long-term factors relate to later phases of decay in which soil chemistry has a greater effect than either soil biology or the gaseous composition of the burial atmosphere. These factors have been studied both archaeologically[5,47,86] and forensically.[5,85,87]

It has been suggested by Mant[86] and Mann et al.[30] that, in the short term, up to 2 years, the soil type is not a particularly important factor governing cadaveric decay.[85] However, experimental work conducted using contrasting burial sites[76] has indicated that in addition to factors relating to microclimate and seasonality, depositional conditions including soil do have a marked effect on decomposition rates.[47] Decomposition is accelerated in porous, permeable, and light soils, which allow a relatively free exchange of oxygen and water from the atmosphere, and reductive gases such as carbon dioxide, hydrogen sulphide, ammonia, and methane from the body.

In general, the deeper the burial, the better the preservation of the body.[47] This is a result of a stable, low temperature; poor gas diffusion; and inaccessibility to floral and faunal agents of decay. However, the action of soil pressure in the burial context can warp bones and this has implications for osteometric analysis in both palaeopathology and forensic osteoarchaeology. Quantifying the extent of this phenomenon is, however, virtually impossible.

Mant[85] observed that a corpse buried and surrounded by certain vegetable matter – straw, pine branches – showed more rapid decomposition than others buried without this material. The straw and pine needles introduced additional bacteria that aided decomposition and surrounded the body with a layer of air. It is also thought that the vegetable matter acts as an insulator, retaining the heat produced by decomposition and generating heat through its own breakdown.[85]

Temperature

Ambient temperature has a profound effect on cadaver decomposition, and in tropical climates rigor may be complete in 2 h while cold will cause it to persist. Bodies sunk in cold water will retain rigidity for a long time, as cold water tends to retard putrefaction. Temperature is a major factor because the microbial processes that occur both internally and externally in a corpse will be affected by this.[30] When temperatures are warm, human decomposition has been documented to occur within 4 min.[31] Contrary to this, at cold temperatures these processes usually begin after 4–7 days.[85] At temperatures below $-5°\text{C}$, decomposition is prevented, as both enzymatic and microbial action will be halted. In the event of death being caused by viral or bacterial infection, not only will the body temperature be higher but bacteria may be widespread throughout the cadaver and hasten postmortem decomposition.

The effect of temperature varies with latitude, season, and depth of burial. In climates where the ground freezes in winter, the burial environment during that period is one of preservation rather than decomposition. Data on putrefaction rates in a temperate climate for a cadaver of average physique have been supplied by Mant.[47,85,87] The onset of putrefaction does not appear for some 36 h in an unrefrigerated body, while in cold but not freezing temperatures the first signs of putrefaction do not appear for 5–7 days. In summer weather putrefactive changes may be pronounced after 24 h.[85]

Moisture

Natural mummification due to rapid drying of the tissues is well attested in both the archaeological[42] and forensic literature.[8] In temperate climates it usually occurs when there is a good air flow and does not usually occur in bodies that have been buried. Desiccation of a substrate to below a critical threshold leads to inhibition of microbial activity but usually does occur when some autolytic and putrefactive change has already occurred – thus the tissue will have been subject to both chemical and mirostructural change prior to dessication. Rehydration of tissue and subsequent histology can reveal these changes.

Contrasting Depositional Environments (Soil Burial vs. Surface Exposure)

The impact of different environmental conditions (buried, surface-exposed, and water-deposited remains) on entomological activity has long been of interest.[88–90] While the depositional environment will affect the rate of decomposition, and in some cases lead to a stasis in breakdown, generally all bodies follow the same basic sequence of decay. Forensic taphonomists have produced a classification of decay sequences that can be applied to a broad range of environmental situations.[10,91]

Few depositional contexts allow for the domination and mutual exclusion of either putrefactive decay or the action of insects. This is particularly evident in the case of surface exposure and shallow burials. Even bodies in confined spaces (e.g., a car with closed doors/windows) will be accessible by a varied insect population. Bass[91] gives a detailed summary for the summer decay rates for a body exposed on the surface at the Anthropological Research Facility at Knoxville, Tennessee.[91]

First Day (Fresh)

In addition to the fly activity, early external signs of decompositional change are the colouration of major veins under the skin that turn dark green or blue, and the exudation of body fluids from the nose, mouth, and anus due to early putrefaction of contents of the intestinal tract.

First Week (Fresh to Bloated)

In addition to active maggot activity, including maggot masses under the skin in the regions of oviposition, the skin will show signs of slippage, and hair will begin to detach from the scalp. The discoloration of the veins become more prominent and an odour of decay becomes apparent. The by-products of decomposition include gas products that initially form in the intestines as a result of the rapid decomposition of their contents, and the gut cavity becomes distended. The distension phase is referred to as a ‘‘Bloat’’. Discoloured natural liquids and liquefying tissues are made frothy by the gas; some may exude from the natural orifices, forced out by the increasing pressure of gases. Molds begin to appear on the surface of the body. The average duration of the decay phases of human remains is known to vary according to season, with the bloat phase being most rapid during the summer months compared with spring or autumn.[10]

First Month (Bloated to Decay)

As the skin starts to breakdown the body cavities will rupture, and the subsequent deflation of the corpse following rupture and purging is known as ‘‘Post bloat’’ or decay stage. Insect activity is diminished. If the body is clothed or covered, the soft tissue will decay to expose the underlying bones. If the is not covered, the skin will get dry and leathery, with maggots protected by the dry outer tissues from direct effects of sunlight. If the body is lying supine, the chest cavity (ribs and sternum) will be held together by a combination of the dried skin and connective tissue. Outer surfaces will continue to be colonized by moulds.

The First Year (Dry)

The skeleton will continue to be exposed, and bone will start to bleach in the sunlight.

First Decade (Bone Breakdown)

It is important to point out at this stage that the wooded hillside on the banks of the Tennessee river in Knoxville that houses the Anthropological Research Facility with its continental US climate differs greatly to the maritime climate of the United Kingdom, which itself has many different geoclimatic conditions.[76] Much of the background work on soft tissue decay and other related factors of interest in forensic cases have been addressed to bodies on the ground surface. Buried bodies are contained in a much less predictable decay environment. Within the less complicated field of material biodeterioration, it is still difficult to produce systematically replicable results for the burial of materials in soil.[92]

The Microclimate Associated with Human Decomposition

The concept of the micro-environment (microcosm) of the grave has been explored by various researchers, with the human body seen as a major nutrient source for microorganisms.[76,93–95] In particular, the decomposition of the body is seen to affect the survival/decomposition of associated death-scene materials. The chemical and biological interactions of a body, with associated materials in a specific soil, are very difficult to model in a realistic manner. The result is that we can describe what we know in a specific set of circumstances; it may be possible to predict general trends within a specific soil type (provided factors of soil moisture and microbiology are largely similar) but it is unlikely that valid prediction can be made between widely different geographical regions or differing burial situations.

Soil conditions at different burial sites have a marked effect on the condition of the buried body, but even within a single site variation can occur; the process of soft tissue decomposition modifies the localized burial microenvironment in terms of microbiological load, pH, moisture, and changes in redox status.[76] The tissues become increasingly liquid, and in the case of a body buried directly in the soil, a mucus sheath will form around the corpse consisting of liquid body decomposition products and a fine silt fraction from the soil.[76,96]

The formation of a highly concentrated island of fertility, or cadaver decomposition island (CDI), is associated with increased soil microbial biomass, microbial activity (C mineralization), and nematode abundance. Each CDI is an ephemeral natural disturbance that, in addition to releasing energy and nutrients to the wider ecosystem, acts as a hub by receiving these materials in the form of dead insects, exuvia and puparia, faecal matter (from scavengers, grazers, and predators), and feathers (from avian scavengers and predators).[95]

Differential Decomposition

The biochemical and microbiology processes that occur on and within a human corpse are very complex. The shorter the timescale between interment and recovery, the more likely soft tissue is preserved. However, it should not be assumed that the presence of extensive soft tissue will indicate a recent death, as in specific burial environments soft tissue remains can be preserved for thousands of years. A skull recovered during commercial extraction of a peat bog in Cheshire during 1983 was examined by a Forensic Science Service pathologist, who noted intact hair and skin, an identifiable eye ball, and pultaceous matter inside the cranial vault. Police suspicions were roused by these well-preserved human remains since a long, unsolved crime was being investigated. However, archaeological dating techniques identified the skull as originating from around the third century A.D.[97] Similarly, the example of Lt. Col Shy killed and subsequently embalmed during the American Civil War is a further cited case.[42]

Before consideration of particular circumstances that have led to soft tissue preservation over long timescales, it is necessary to consider the nature of soft tissue that has been subject to partial decomposition. It was originally thought that natural mummification and the formation on hydrolysed but not oxidized fat (adipocere) were mutually exclusive processes. However, it has been demonstrated by Mant[14] that both tissue types can be encountered from cadavers in forensic cases.

Soil, with its varied oxygenation, water content, redox potential, ion exchange capacity, and pH variation, as well as the nature of the body, its biochemistry, fat content, cause of death, time interval between death and burial, whether it is clothed or unclothed, wrapped in a polythene sheet, buried shallow or deep, affect forensic analysis and interpretation of data obtained from human remains. In short, it is very unwise to draw direct predictive parallels between one specific case and another. Experimental work can indicate general trends for the specific parameters tested.[76]

Mant[86] observed significant retardation of decomposition in clothed bodies buried directly in the soil without a coffin. Clothing will partially negate the effects of the general soil environment and delay the process of decay. Textiles around the body also impede access of burrowing carrion scavengers. Even after 2 years in shallow graves, those parts of the body covered by clothing frequently showed good preservation. It was also observed that adipocere formation was uniform and putrefactive liquification rare and that muscle tissues and muscle attachments to bone were still well preserved in areas such as the thighs and buttocks where the thick layer of fat was only in the process of hydrolysis and hydrogenation.

It has been suggested that, in general, the time required for the decomposition of corpses takes between 3 and 12 years.[98,99] If conditions are less favourable, the time delay is much higher and can be hundreds or even thousands of years before skeletalization of a human corpse[100] Following putrefaction, the decomposition process continues through liquefaction and disintegration, leaving skeletonized remains. Skeletonization proceeds until eventually only the harder, resistant tissues of bone, teeth, and cartilage remain. Bacteria and fungi aid to skeletonize the corpse.[100]

Both forensic pathologists and archaeological scientists are familiar with depositional environments that can retard the decomposition of soft tissues over long timescales.[13] The desiccation of tissue below thresholds for microbial activity will lead to preservation. This natural mummification is well documented in the forensic sciences literature.[13,15] Mummified bodies usually exhibit a marked reduction in tissue bulk, often accompanied by darkened skin resembling dried leather. Since tissue water loss is from exposed surfaces, there is often a difference in water content between core and peripheral tissues. This was directly recorded by Janaway and Wilson (publication being written) in recent experimental burial of pig cadavers in the costal desert of Southern Peru. After 2 years of burial, directly in the sand, the exterior tissues had formed a hard, desiccated layer, while interior of the body core remained moist. This differential desiccation is also observed in archaeological mummified bodies from the same region. It should be noted that desiccated tissue rarely has not been subject to putrefactive change prior to the moisture threshold dropping to the point where microbial activity is significantly inhibited. In extreme cases, a skeleton may be articulated because of intact ligaments and covered by a dried out skin of once-liquefied tissue lacking any residual morphological structure. This has been observed in both archaeological bodies and is also well documented at the Anthropological Research Facility at Knoxville, Tennessee.[12,73,101] Owing to surface area/volume effects, different parts of a body placed in desiccating environment will lose water at different rates. For instance, it is not unusual to observe well-developed mummification of hands, feet, and limbs while the trunk is still subject to major putrefactive change. An example from recent casework is instructive. The body of a young woman, who had been killed by blunt force trauma to the head, had lain in a cool, dry cellar for a number of months. The body was partially clothed and there had been considerable blowfly activity. While adult flies were actively hatching from the pupae within the cellar, there was no longer significant larval activity in the body. The head had been covered, and thus excluded the blow flies, although there was considerable putrefactive change accelerated by the trauma. The relationship between trauma and soft tissue decay was documented by Mant over 50 years ago.[87] The body was naked from below the waist, and the exposed uterus and internal tissues had been destroyed because of extensive feeding by fly larvae. The exposed limbs and lower body hair was desiccated and well preserved in an advanced state of mummification. Thus at the time of autopsy this single body exhibited massive tissue loss from putrefactive change, massive tissue loss due to the feeding of a maggot mass, but relatively intact tissue due to partial desiccation.

Under damp conditions, hair is readily attacked by keratinolytic microorganisms. Under dry condition human hair it is often preserved over long timescales, while still liable to attack, but usually not total destruction by insects such as the Dermestid beetle (*Anthrenus* spp. or Clothes moth larvae (*Tineola bissiella*).[40]

In addition to desiccation, low temperature regimes will inhibit microbial activity and therefore reduce putrefactive change.[29] Bodies have been preserved by both freezing due to the natural environment as well as the use of domestic freezers. Care must be taken to distinguish between tissue that is largely hydrated but frozen, and tissue that has desiccated because of the cold, dry conditions that are, for instance, found in many mountainous and arctic regions. Freeze-dried human tissue has survived over hundreds of years as is the case of the Greenland mummies and frozen bodies, buried in the 1840s, that have been exhumed from the Canadian arctic. In this case, at a macroscopic level the bodies exhibited good levels of soft tissue preservation, but microscopically little histological structure remained.

Conclusion

Clearly the human microbiota has a role to play in decomposition of the host. In fact, the indigenous microorganisms ultimately lead to the demise of their host. The microorganisms that were once classified as the indigenous human flora then persist within the environment and become free to colonize another host. The ‘‘microorganism and human cycle’’ then begins again.

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