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Waxing Grave About Adipocere: Soft Tissue Change in an Aquatic Context*

ABSTRACT: When postmortem environmental conditions are "just right," according to the "Goldilocks Phenomenon," soft tissues (and associated fatty acids) are converted into and preserved as adipocere. To better understand this conversion process and the development of adipocere three human cadavers were immersed in outside, water-filled pits for over 3 months to observe adipocere formation in an underwater context simulating actual field conditions. Recordings of environmental conditions showed that temperatures were between 21°C and 45°C, a range sufficient for the growth of *Clostridium perfringens*. Chemical analysis of liquid and tissue samples revealed an increase in palmitic acid and decrease in oleic acid. This study tracked the remarkable gross morphological changes that can occur in human bodies subjected to an aquatic postmortem environment. The results support the "Goldilocks Phenomenon" and substantiate previous findings that the presence of bacteria and water is crucial for adipocere to form.

KEYWORDS: forensic science, forensic anthropology, adipocere, postmortem interval, saponification, inhumation in water, fatty acids

Given proper conditions in the postmortem environment, a body's soft tissues may become preserved rather than decompose. The preserved tissue, adipocere, is produced through saponification, a hydrolysis of the body's fatty acids (1). This process usually occurs "under virtually anaerobic conditions in which human fat is converted into a complex of saturated fatty acids by a great variety of bacterial species occurring in and on the decomposing body" (2).

Knowledge of adipocere formation is still limited, even though it has been studied since the late 18th century. Previous studies have analyzed adipocere for its chemical components and its microscopic properties, have investigated the differential formation of adipocere, and have attempted to recreate adipocere in the laboratory, or have tracked its development in nonhuman subjects (3–24). To date, no one has explored its unique formation in human cadavers subjected to simulated field conditions from the onset of saponification to the complete formation of adipocere.

In this actualistic study, human cadavers were submerged in water-filled pits at the Anthropological Research Facility (ARF) in Knoxville, Tennessee (25). This study approximates typical field conditions for underwater decay by providing the basic conditions most conducive to adipocere formation: presence of skin and human fatty tissue, a moist, warm, anaerobic environment, and the presence of putrefactive bacteria (4).

The necessity for such research stems from a need for more comprehensive and diachronic investigation of the transformation of human soft tissue into adipocere. The current literature contains a number of specific case studies (21,23,26–29), although no one offers results from a long-term study that visually tracks and observes the gross morphological changes of adipocere development. This study is significant because it provides detailed, descriptive observations taken over a 3-month period which may be compared with cases involving aquatic deaths in order to better assess the postmortem interval.

Adipocere Formation: Chemistry

In 1789, Fourcroy investigated the chemical composition of adipocere during exhumations of interred bodies from the *Cimetiere des Innocents* in Paris (2). He suggested the bodies had not completely decomposed but rather transformed into a fatty, waxy tissue; hence the term *adipocere* (French. *adipocire* <Latin: *adeps*, fat+*cera*, wax). He noted that adipocere was most prevalent in the fatty regions of the body such as the cheeks or breasts, and there was remarkable desiccation of the internal viscera.

It was not until the early 20th century that Ruttan and Marshall (3) quantified the components of adipocere by analyzing samples taken from pigs. They concluded that adipocere is:

> . . . composed almost entirely of the insoluble fatty acids [palmitic acid 67.52%] left after the slow hydrolysis of the fats in wet ground. The protein matter has entirely disappeared and the glycerol, soaps, etc, resulting from the hydrolysis have been carried away in aqueous solution. The insoluble hydroxystearic acids [15.8%], which are so characteristic of adipocere, are probably derived from a portion of the oleic acid in the original fat by hydration (3).

The process of adipocere formation, or saponification, begins in neutral fat (i.e., adipose) and is initiated by intrinsic lipases, which degrade the triglycerides into fatty acids (30–35). The cell membrane of a fat cell, or adipocyte, is a lipid bilayer made from glycerol, fatty acids, phospholipids, and proteins. It functions to maintain equilibrium with its external environment, especially when exposed to an excess of water. In such a condition the cell

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will absorb water until it ruptures subsequently releasing its fatty contents. The exposed fatty acids are then hydrolyzed and hydrogenated by chemical decomposition facilitated by degradative anaerobes, such as *Clostridium perfringens [welchii]*, which convert the neutral fat into hydroxy fatty acids by secreting toxins containing proteases and phopholipases that subsequently destroy the cell membrane. The bacterial action creates ammonia-rich waste that contributes to forming an alkaline environment. This unsuitable environment will inhibit continued bacterial growth, arrest putrefaction, and may either kill the bacteria or stimulate sporulation (4,11,35–38).

The completely saponified adipose tissue, called adipocere, tends to be "firm, grayish-white and of a wax-like consistency" (27), resemble lard or "butter" (2), and has been described as friable, caseous, homogenous, cheesy, and crusty. Additionally, if a moist, adipociferous body is allowed to desiccate it can develop a superficial yellowish-gray hue and leather-like texture (39). In this state, the corpse may appear larger than its antemortem size due to the expansion of fat tissue that has become dense and thickened (5). Furthermore, as water is extracted from the tissues and utilized for hydrolysis, the viscera may appear shrunken. In this condition, the body may be preserved for an indefinite period of time due to adipocere's stability as a result of the high melting point of the newly formed hydroxy fatty acids (e.g., saturated palmitic acid) (27,40–42).

Following such pioneering efforts by researchers like Ruttan and Marshall or Mant and Furbank, several others have continued to investigate the chemistry of adipocere and confirmed that as the amount of naturally present oleic acid decreases the amount of palmitic acid increases (2–6,9,10). Many agree this conversion is facilitated by bacterial enzymatic activity. Saito (7) states it consists mainly of saturated fatty acids with a large amount of hydroxy fatty acids. Using gas chromatography and mass spectrometry, Takatori and Yamaoka (9,10) identified that 10-oxo-hexadecanoic acid and 10-oxo-octadecanoic acids complement the primary and secondary components of 10-hydroxyoctadecanoic [hydroxystearic] and 10-hydroxyhexadecanoic [hydroxypalmitic]; see also: (43,44). Gotouda et al. (12) have shown that these 10-hydroxy and 10-oxo fatty acids are present in human adipocere but absent in the original adipose tissue.

Adipocere Formation: Variables

It has been shown that adipocere forms in various environments. These include areas such as bathtubs, ponds, lakes (28,39), and oceans (26) see also (45), as well as enclosed locations such as underground burials (20,40,46), cave burials (47), and inside caskets (5,41,48). Adipociferous bodies have also been found wrapped in plastic or trapped in bogs (13). Thus, as both Mant and Furbank and Evans (4,5) agree, the basic requirements for the formation of adipocere are a moist or aquatic environment (i.e., complete or partial immersion), warm temperatures, intrinsic bacterial enzymatic action, and adipose tissue. Mellen et al. (21) add that anaerobic conditions also facilitate formation. O'Brien (39,49) also stresses the importance of temperature and moisture in his description of the "Goldilocks Phenomenon." It states that adipocere tends to form in intermediate ambient conditions, or when they are "just right." In other words, tissues will desiccate if the conditions are too dry and cold; whereas, if conditions are too wet or hot then the body may macerate or possibly liquefy.

Other variables, which affect adipocere formation, include such conditions as relative humidity or pressure (e.g., if the body is discovered in a deep underwater location, see (19). Evans (5) notes that when buried on days with fog or haze present bodies are more likely to develop adipocere than those buried on days of pleasant weather. Additional environmental factors include: a suitable environment for putrefactive bacterial growth, the effects of water current such as a stagnant pond or moving river (50,51), the chemical composition of the water like in a chlorinated swimming pool (see (15)) or salt water (14,19,52) or industrial waste water (27), seasonal limnological fluctuations (39,53), and predation by insects, marine animals, or fish (54) which can contribute to tissue loss on the adipociferous corpse (17,22). The presence of clothing also tends to promote adipocerization in bodies because it protects the body from the elements (4,5). Additionally, articles found in direct association with bodies pulled from water (e.g., rope, plastic, blankets, concrete blocks, battery cables, barbecue grill, a tire wheel) tend to inhibit adipocere formation (28). Furthermore, personal features of the decedent, such as age, sex, antemortem weight and height need to be considered when interpreting an adipociferous corpse (40).

It is extremely important to recognize that adipocere formation is affected by a wide variety of factors and conditions. Therefore, to more effectively predict the postmortem interval by an examination of the state of adipocere development, all variables must be tested. Whereas a comprehensive study would test all of these influential variables, logistical, physical, and theoretical constraints lead to only a few highly salient factors being observed in the present study. The purpose of this study is to observe the development of adipocere in bodies submerged in water and to identify significant environmental factors that affect the development of adipocere.

Materials and Methods

This study was conducted from October to January at the University of Tennessee's ARF in Knoxville. Three holes measuring *c*. 2 m long and 1 m in width and depth were excavated at least 1 m apart from each other and in proximity to a water source (i.e., an underground pipeline, originating from the Knoxville Water Treatment Plant, with service through an aboveground faucet) (25,49). The first two were lined with clear, polyethylene plastic to limit water loss. The third was not, in order to determine any effect the plastic might have on adipocere formation and also because of the argillaceous nature of the soil matrix.

To facilitate retrieval and documentation, each body was placed on a support tray measuring *c*. 2 m long and 1 m wide and constructed from a frame of untreated pine wall studs connected by 16 penny nails. The body rested on and a bed of utility-wire fencing attached to the wood with 2″ (5.08 cm.) roofing nails. Four concrete blocks were placed on the floor of each hole to support the tray when and if it submerged. To aid in retrieval, lengths of rope were attached to both ends of each tray and tied off above ground. Following construction of the holes and trays, each hole was filled with water.

Three unautopsied, adult male, human cadavers, either unclaimed or donated to research, were acquired from the Nashville Medical Examiner's Office. Each had been in a morgue cooler for a period of about 3 months. Upon arrival at the ARF, body bags were opened individually to limit ovapositioning before submersion. Each unclothed cadaver was placed on a support tray in a supine position with the arms to the side and legs slightly spread apart. Initial observations were conducted before submersion and outlined below.

Hole 1 contained the body of an adult, white male (weight = 85 kg, height = 178 cm.) who died from multiple blunt traumatic injuries sustained in a fall and had extensive open trauma across the head. The neck and upper torso were moldy, pockmarked, and bruised

especially around trauma-affected areas. Slight skin sloughage was noted at various sites across the body. The soles of the feet and the palms of the hands were already extremely wrinkled.

Hole 2 contained the body of an adult, white male (weight = 78 kg, height = 172 cm) who died from a gunshot wound resulting in open trauma to the left lateral and posterior aspects of the head. No other trauma or pathology was noted. The abdomen appeared partially bloated with skin slippage and wrinkling present on the hands and feet.

Hole 3 contained the body of an adult, black male (weight = 100 kg, height = 175 cm) whose cause of death was not indicated on ARF case records. The body was in a good physical state with no apparent signs of trauma or pathology. The abdomen appeared partially bloated with skin slippage and wrinkling present on the hands and feet.

Observations of water loss and gross morphological changes and measurements of water and air temperatures were made about every 2 days from the start of the project (i.e., October 20) and eventually slowed to about once a week until the end of the project (i.e., January 22). Temperature was measured using a Universal Enterprises T220 Fahrenheit food thermometer (Universal Enterprises, Beaverton, OR). Photographic documentation was conducted using an Asahi Pentax K1000 camera (Pentax Imaging Co., Golden, CO) and SMC Pentax-A 1:2 50 mm lens (Pentax Imaging Co.) using Kodak Ektachrome Elite 200 35 mm daylight film (Eastman Kodak Co., Rochester, New York) for color slides. To avoid aquatic distortion during photographing the tray was lifted out of the water if necessary.

In order to detect fatty acid concentrations and the presence and type of microbes living in, on, or around the decomposing body liquid and soft-tissue samples were extracted from the cadavers using a Becton Dickinson 10 cc. syringe (Becton Dickinson, Franklin Lakes, NJ) accompanying a Becton Dickinson 18G 1/2 Precision Glide needle at *c*. 5, 9, and 12 weeks after initial immersion. Each sample was subjected to a Bligh and Dyer test where an organic solvent (i.e., chloroform and methanol, with a phosphate buffer to keep the pH neutral) was added to recover as much lipid material as possible. This test produced both residue and extractable lipids. The residue was put through an acid hydrolysis process, which allows a hydroxy fatty acid methyl ester to be separated. The extractable lipids were placed in a silicic acid column and separated into classes by polarity. Chloroform, acetone, and methanol were all used to isolate the neutral lipids, the glycolipids and the polar lipids, respectively. The neutral lipids contain such items as steroids, hydrocarbons, diglycerides, triglycerides, and free fatty acids. Using thin layer chromatography, the free fatty acids were isolated and analyzed. The polar lipids were extracted and identified with gas chromatography.

Results

In the following section, each hole is designated and referred to by the corresponding body that occupied it (e.g., body in the first hole = Body 1). Following immersion, Body 1 completely submerged within 30 min. Body 2 floated high on the water surface with exposure of the anterior portions of the thighs, upper arm, abdomen, thorax, neck, and head along roughly a coronal plane. Body 3 floated slightly lower than Body 2 with exposure of the face, anterior portions of the shoulders, upper arms and thighs, and the lower portion of the abdomen.

In the first week, all holes had leaves scattered across the water surface due to the season (i.e., Fall). Body 1 was unremarkable and unchanged. Bodies 2 and 3 had not submerged (see Fig. 1). In the second week, Body 1 had a softening of the flesh and the water was murky and cloudy. The body emitted a pungent odor of decay. Body 2 remained floating with noticeable effects of early putrefaction: distended belly, scrotum, and penis, red and purple discoloration. The lateral aspects of the body were more exposed with bloating and slight water loss from the hole. Ovapositioning by bottle flies was noted in the oral and nasal cavities. The soles and palms were more wrinkled and continued to peel. When the mouth was opened to observe dentition a red, viscous liquid seeped out. Body 3 was covered with a thin layer of sediment that had accumulated from the water being disturbed during immersion. Between the dried, cracked areas of the sediment on the abdomen, green hues were observed on the skin. A red liquid also seeped out of the oral cavity upon inspection of the dentition. The hands and feet were still wrinkled.

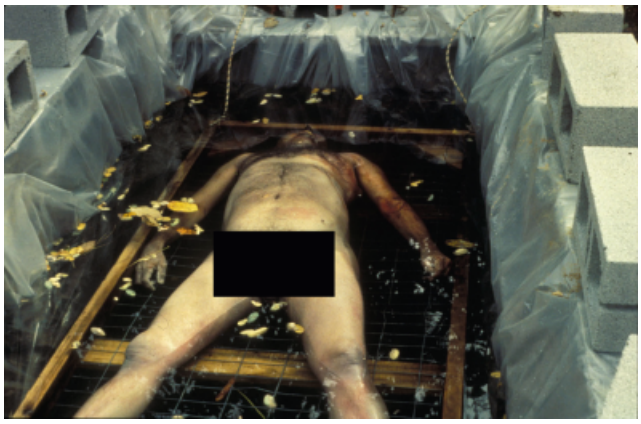

FIG. 1—*Body 2 on first day of immersion.*

In the third week, Body 1 remained submerged. Body 2 remained floating and putrefaction continued. The discoloration on the arms, neck, and upper thighs resembled bruising. In Body 3, puddles of a whitish, milky, thin, oily film surrounded the body making the water appear thicker and denser. Also along the exposed body contours was an oily film. Odor was present in all holes.

In the fourth week, Body 1 remained submerged and a slightly opaque film had collected among the leaves littering the water surface. Body 2 remained floating and displayed continued insect activity. Beetles were crawling over the exposed torso. The exposed chest and abdominal tissues had discolored further with an evolution of patchy, moldy clusters of brown fungal growth (see Fig. 2). The hands and feet remained wrinkled and peeling. Body

FIG. 2—*Body 2 during fourth week.*

3 remained floating with the presence of flies but no visible ovapositioning. The water increased in cloudiness to almost total opacity and was viscous to the touch. Tissue decay was noted. Leaves littered the surface.

In the fifth week, Body 1 remained submerged and did not show any signs of putrefaction. A green, slimy algal-like growth formed a thin, opaque film on the surface of the water. Body 2 remained floating, and the eggs deposited in upper neck and facial regions had developed into a mass of maggots. These appeared slightly lethargic, but others were writhing under thin bubbles of flesh across the body. Beetles were absent. Without moving the body, it was observed through the water that skin from the underside near the middle back was sloughing but not fully disarticulated. The epidermal tissues just above and below the water were wrinkled and thickened. The color of the body closer to the water level had changed from the original pigmentation to a pale yellow color. A general loss of color was noted, although the exposed portions of the body (i.e., upper chest, lower abdomen, and upper thighs) were darker red and brown with a hardened, mummified texture. Sloughed soft-tissues and fluids seeping from the body collected and formed a scum on the water surface around the body. Body 3 was positioned high in the water due to considerable bloating and abdominal distention. A slimy mat of oily, thick algal-like matter covered the water surface. This feature is similar to what was observed in the first hole. Few insects were observed. There was a slight lightening or graying of the soft-tissues exposed.

In the sixth week, Body 1 was unremarkable and remained submerged. The water had a denser appearance. Body 2 remained floating. The mouth had flared lips and held numerous maggots. Other exposed facial regions remained unaffected. The previously darkened regions of the body above the water had become lighter in color. The brown fungal growth changed in color to a paler hue. Tissue in the "water-level zone," the area of the skin on a floating body that runs *c*. 8–9 cm above and below the surface of the water, was more wrinkled, billowed, and warped with a yellowish-white color. *Cutis anserina* ("goose-pimply" flesh) was apparent in this zone and on the exposed shoulder and upper thigh regions. Body 3, with the exception of being distinctly lighter in skin color, had no remarkable change. A small area on the right arm near the elbow displayed evidence of *C. anserina*.

In the seventh week, Body 1 remained submerged. The anterior portion of the body was completely covered in a thin mat of green algal-like slime, which also covered the plastic walls of the hole. The pungent odor of decay was still present. The body was still corpulent with no signs of bloating or tissue loss. The tissue was soft, wet, and soggy. Body 2 remained floating with continued yellowing of the lateral portions of the body (see Fig. 3). The skin appeared to be more wrinkled and rippled in the "water-level zone" (see Fig. 4). The mouth remained flared. Many maggots had migrated into the water. The chest and lower abdomen were dried and displayed brown patches, and an orange mold-like growth had appeared. The tissues of the hands and feet were still wrinkled and peeling. Body 3 had no remarkable change but was notably desiccated. Intermittent heavy rains had fallen during the previous 2 weeks, subsequently churning up the water and disturbing the surface films and algal growths that had accumulated on the water surfaces.

In the eighth week, the water visibility in the first hole was notably opaque. Body 1 remained submerged with no remarkable changes. Body 2 was still floating. Gross changes made tissues in the "water-level zone" appear warped and crumpled, especially around the abdominal region, waist, and shoulders. Any exposed tissue had desiccated. The penis, scrotum, and abdomen had deflated. The extremities were yellow and whitish-gray. Maggots still worked in the oral cavity even though it had slightly submerged. Body 3 continued to desiccate without remarkable change. The exposed body was light brown and beige in color. A thin film remained on the surface of the water.

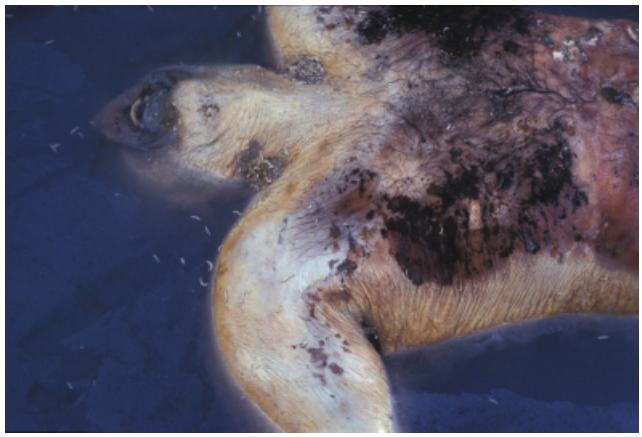

FIG. 3—*Body 2 during seventh week.*

Through the ninth and 10th weeks, Body 1 had no remarkable changes and remained submerged. Body 2 remained floating and unchanged in general form and structure, and had little maggot activity. The bright orange mold-like growth remained on the upper chest. Body 3 remained deflated with no remarkable changes. Bodies 2 and 3 remained floating.

In the 11th week, Body 1 had no remarkable changes and remained submerged. Body 2 remained unchanged in general form and structure. The orange mold-like growth had spread across the chest cavity to the upper shoulders and upper thighs. Although slightly lower, the body remained floating. Body 3 remained floating and deflated.

In the 12th week, Body 1 had no remarkable changes and remained submerged. Above the water, Body 2 was covered with the orange mold-like growth on top of patchy, brown, mummified tissue. Along the "water-level zone," it remained wrinkled and warped, bordered by *C. anserina* (see Fig. 5). Body 3 remained floating and deflated. Certain areas of the arms and upper thighs appeared wrinkled with slight *C. anserina*.

After a period of 3 months, Body 1 never surfaced, appeared soggy and covered in an algae-like slime, and had little gross morphological change to the soft-tissues other than a general color loss and early indications of macerated tissue. In contrast, Bodies

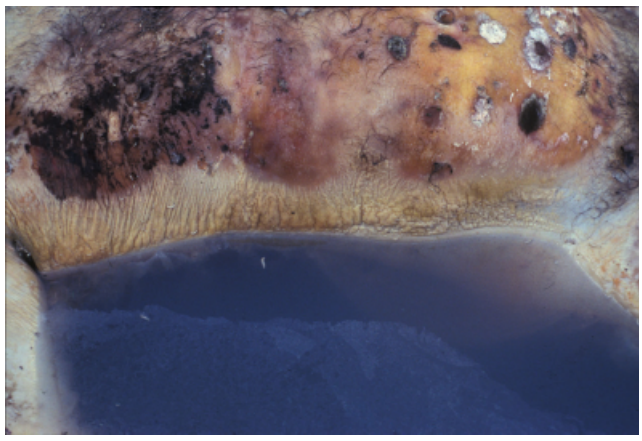

FIG. 4—*Body 2 during seventh week, with close-up of water-level zone.*

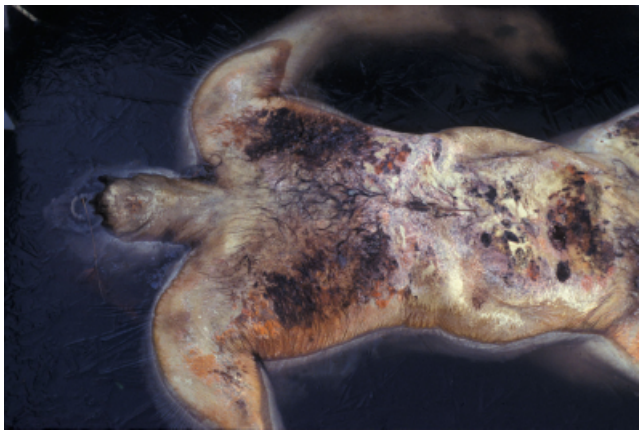

FIG. 5—*Body 2 during twelfth week.*

2 and 3 had noticeable color loss, subsequent desiccation of exposed tissues, and extreme gross morphological change to soft-tissues in the "water-level zone." The tissue, which had formed on Bodies 2 and 3, in respect to its form, structure, and texture, is considered to be adipocere. Confirmation was substantiated by chemical analysis. The analysis of extracted tissue and liquid samples indicated that concentrations of palmitic acids had increased while concentrations of oleic acids had decreased. Furthermore, a polar lipid fatty acid profile identified the absence of *C. perfringens [welchii]*, the primary bacteria responsible for adipocere formation, in the body in the first hole.

Discussion

In the present study, none of the bodies followed the typical stages of underwater postmortem activity that has been observed in comparable studies such as: floating, sinking, putrefaction, subsequent refloatation, differential decomposition, and the final sinking (22,24). In particular, following immersion Body 1 sank and remained underwater for the length of the study. When raised, a mat of green, algal-like slime was observed covering the body's ventral surface. As there was open trauma around the head and neck, and indication of some decay before immersion, it is likely this body provided a nutrient-rich environment for algal growth, which inhibited the bacterial activity required for saponification (55). Furthermore, the abdomen never bloated, and the body did not progress through the expected decompositional stages as described by Knight (56). At the last formal observation, there were no signs indicating that the soft tissue had saponified into adipocere. Instead, it appeared to have macerated, though without significant loosening or disarticulation of soft-tissue.

Body 2 had the most notable changes. These were easily observed because the body remained floating and generally corpulent for the extent of the project. In this position, the body was subjected to differential decomposition, both above and below the "water-level zone." Additionally, a bright orange growth evolved on the cadaver's shoulders and chest. It was identified as *Fusarium* sp., considered a contaminant occasionally involved in skin and systemic infections in severely debilitated hosts (57–59).

In addition to fungal growth, other notable transformations were changes in color and appearance of the soft tissues. Body 2 had noticeable color loss. In only 3 weeks after immersion body color changed from dark pink skin tones to reddish-brown to dull yellow and white. The first signs of characteristic adipocere tissue were noted in the "water-level zone" around the sixth week, when *C. anserina* was observed speckling the body in areas around the shoulders and upper arms as well as the lateral aspects of the thoracic cavity and down to the upper thighs. After the emergence of this goose-pimply texture, the tissues became thicker and denser, exhibiting ripples and billows perpendicular to the water surface. The tissues above the "water-level zone" had become dry and hardened, while tissues below were soggy and loose with no gross tissue loss or bone exposure.

Body 3 in the third hole progressed through similar stages as Body 2, but with an absence of maggot activity and fungal growth. The changes occurring at approximately the same time, as Body 2, included: the rippled and billowed tissue texture in the "water-level zone," advanced *C. anserina*, desiccation of exposed tissues, while those below the water appeared soggy and loose. Body 3 also had noticeable color loss; but unlike Body 2, the dark brown skin color generally faded as it approached the final yellowed color instead of darkening further (as it might if fungal growths appeared). Although the body was very dark brown when removed from the body bag, during putrefaction small patches of the outer epidermal layers had peeled away to reveal lighter pink tissue below. After being in the water for 5 weeks, the body exhibited further color loss, a condition that Mann et al. (34) state is typical in blacks during decomposition.

Therefore, based on the combined evidence of gross morphological tissue changes and chemical analyses it is concluded that after 3 months adipocere had formed on the bodies in the second and third holes but not in the first. There are many factors that contribute to adipocere formation or the lack thereof; the foremost being a certain range of temperatures. For example, Cotton et al. (27), cite a case report where adipocere was found on bodies in water exceeding 21°C; whereas in Payne and King's (22) study of the formation of adipocere in pigs, peak water temperatures reached close to 27°C. Additional studies suggest that while adipocere does form in a wide range of water temperatures, the rate at which it develops varies. Mellen et al. (21) show that in lukewarm water (15–22°C) adipocere can form in as little as 2–3 months after immersion. Sledzik and Micozzi (60) suggest that in cold water (4°C), adipocere will form between 12 and 18 months. Furthermore, Simonsen (26) discovered that adipocere could develop after only a few weeks in very warm water (*c*. 22°C according to (61)).

In another study (19), 15 bodies were recovered from a shipwreck in cold seawater (10–12°C). Examination showed that the first five bodies recovered (two on the second day and three after 25 days) had the expected bloating, marbling, and slippage, but a body recovered at 38 days exhibited subcutaneous adipocere formation on the face and abdomen. Two more cadavers were recovered at 68 days. The skin on these bodies was easily removed, and underneath were large confluent patches of adipocere. At 109 days, three more bodies were recovered in which all the cutaneous and subcutaneous tissues had been transformed into adipocere with an outer friable crust. The internal organs also displayed some adipocere formation. The lower limbs of the cadavers were partially skeletonized. The final four corpses were recovered 433 days after the shipwreck. Three of these individuals, found in an open cabin, were skeletonized with a few patches of adipocere tissue. The fourth was recovered from a closed cabin and the cutaneous and subcutaneous tissues were completely saponified. Although it appears that water temperature determined the rate of adipocere formation, this study suggests that the water current (most of the bodies that had adipocere present were in enclosed cabins), the presence or absence (as was the case) of flora and fauna, and the type of water (salt, fresh, etc.) also contributed to the formation of adipocere.

TABLE 1—*Stages of adipocere formation on a body floating in water for 3 months.*

Stage of Adipocere Development	Postimmersion Interval (Weeks)	Morphological Changes
Stage I	0– ~ 4	Float, bloat, ovapositioning and hatching
Stage II	~ 4–6	Early: insect activity, skin sloughing, cutis anserina, residue formation on water surface
Stage III	~ 6–8	Early: adipocere formation, microbial growth, color loss Increased: insect activity, skin sloughing, cutis anserina, residue formation on water surface
Stage IV	~ 8–10	Increased: adipocere formation, microbial growth Advanced: insect activity, cutis anserina, color loss, residue formation on water surface
Stage V	~ 10–12	Advanced: adipocere formation, microbial growth Reduced: insect activity

The variables inherent in the present study could not be completely controlled. These include: the cold storage of the bodies before immersion and its effect on decay, ambiental conditions, and the presence or absence of certain flora or fauna. Nevertheless, field conditions were approximated and certain variables were explored. For instance, the addition of polyethylene plastic to the first and second holes provided a somewhat controlled and artificial context. This impenetrable barrier not only was used to prevent extensive water loss, even though water was periodically added to all three holes (i.e., manually and through the effects of precipitation), but also to provide an additional environment by which adipocere formation could be studied and compared. The results of the study showed that the body in plastic-lined Hole 1 did not form characteristic tissue resembling adipocere, but the body in plastic-lined Hole 2 did. In contrast, the body in the third hole, which was unlined, also formed adipocere. Therefore, it is reasonable to conclude that the plastic did not play a significant role in adipocere formation.

Particularly for the study presented here, fluctuations in air temperature ranged from below freezing to as high as 24°C. Therefore, it is suggested that the temperature diversity during this study contributed to keeping the bodies corpulent. In colder temperatures, bacteria responsible for adipocere development slow down their enzyme production but continue to survive. In warmer temperatures, their numbers can multiply so quickly that it is difficult for a body to stay corpulent because decay is so rapid. According to Tomita, the bacterial species *C. perfringens* [*welchii*] will not grow in laboratory temperatures at or below 21°C (54). Bryan and colleagues contend that *C. perfringens* [*welchii*] has an optimum growth temperature of 35–37°C (62). However, Corry (1978) (11) disagrees, explaining the optimum growth temperature for this species reaches about 45°C. In contrast, frozen bodies tend not to exhibit adipocere because of the unsuitable conditions for bacterial proliferation (63). Based on information from these studies, the optimum growth temperature seems to be from about 21–45°C. In other words, when ambient temperatures are lower than 21°C and higher than 45°C, adipocere will not form due to a depression in the rate of bacterial action and enzymatic release. Therefore, the temperature range for adipocere formation has to be just right, what O'Brien calls the "Goldilocks Phenomenon" (39,49), otherwise it will not form.

In this study, during the 3-month period involving 22 observations, mean water temperature was a little over 9°C, ranging from 0 to 18°C. Mean air temperature for the 3-month period was 11°C, ranging from −4 to 24°C. Modes for both sets of temperatures clustered around 7.2°C during which time adipocere formation was most likely occurring. However, in the end only two of the three bodies formed what we classify as adipocere. According to the other line of evidence from which data was collected, results are essentially inconclusive, but do show the typical increase in palmitic fatty acid and decrease in oleic fatty acids characteristically found in adipocere. Furthermore, in the body that did not form adipocere, the polar lipid fatty acid profile revealed the absence of the bacteria *C. perfringens [welchii]*, thereby suggesting why adipocere may not have formed on it.

Based on the results from the current study in which field conditions are approximated (e.g., highly fluctuating temperatures, a stagnant pool of water and the potential for the body to remain in an extended floating state), the following stages of adipocere development are presented as they pertain directly to the morphological changes seen in the Body 2 (see Table 1). In the first stage, following immersion and initial floatation, postmortem events are characteristic of bodies deposited on the surface of the ground: putrefaction (bloating, marbling, and general discoloration), ovapositioning facilitated by the distention of the abdomen and other body parts above the water surface, and the detection of decompositional odors. The second stage is characterized by initial sloughage of exposed epidermal layers, larval hatching with subsequent maggot activity, an early form of *C. anserina*, and residue formation on the water surface. In stage three, there is early microbial growth, and increased insect activity and sloughage of epidermal tissues. Levels of *C. anserina*, color loss, desiccation of exposed tissues, and residue formation are increased. Tissues below the water in this stage may show early signs of maceration with slight disarticulation. The fourth stage is characterized by changes in the "water-level zone." In this area, the tissues exhibit marked yellowing and warped, billowed striations perpendicular to the water level. The formation of this layer indicates that saponification is reaching its end and that adipocere has formed in the body. Furthermore, levels of *C. anserina*, microbial growth, color loss, and desiccation of exposed tissues are more advanced. Tissues below the water in this stage will show advanced signs of maceration with notable disarticulation. In the fifth and final stage, insect activity is reduced, but fungal/microbial growth and desiccation of exposed tissues is advanced. Internal soft-tissues are saponified and characterized by their stability at room temperature. These stages are presented for this particular environment (i.e., southeastern Tennessee during its colder months) and similar environments with seasonal temperature fluctuations.

Conclusion

The saponification of human adipose tissue is a preservation process resulting in a tissue called adipocere. This process should not be confused with normal soft-tissue decay or decomposition because the newly transformed tissue is homogeneous, well structured and, depending on the postmortem environment in which it is stored can remain in a virtually unalterable or stable state. At first, when removed from an aquatic environment, adipocere tends to be soggy and doughy. Over time, if allowed to dry, it will easily

crumble or split apart but not chemically decay further. Many agree that at the adipocere stage, postmortem degeneration is complete (4,5) and thus, interpretation of adipocere can often perplex the forensic scientist when developing an appropriate postmortem interval (e.g., (41)).

This study demonstrates how temperature acts as a major variable in underwater decomposition affecting the rate of adipocere formation. The "Goldilocks Phenomenon" applies in this study because there is a certain range of temperatures for the formation of adipocere. Ambient air or water temperatures cannot be too hot or too cold but "just right." This range falls well within the range for the optimum growth temperature for *C. perfringens* [*welchii*] (21–45°C). It is evident from a comparison of this study with other similar studies, that each environment will have its own requirements for adipocere formation. Further application of the "Goldilocks Phenomenon" suggests each environment has unique requirements that must be "just right" for adipocere to form (39,49). Although limited in sample size, variables, and the range of environments tested, it is possible to say that complete immersion in an aquatic environment and consistent temperatures are not necessary for adipocere to form.

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