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PAPER

ANTHROPOLOGY

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Experimental Adipocere Formation: Implications for Adipocere Formation on Buried Bone*,†

ABSTRACT: Adipocere, or grave wax (adipo = fat, cere = wax), is a distinctive decomposition product composed primarily of fatty acids (FA) and their alkali salts. FA result from the bacterial enzymatic hydrolysis of body fats. Reactions with ammonia and alkali metals originating from body fluids and pore waters of the depositional environment produce alkali salts of FA (soap). Adipocere formation is generally associated with burial of corpses with ample adipose tissue available. No indications that adipocere can form on defleshed remains have been presented in the literature. At the termination of a long-term bone diagenesis experiment, several samples were found to possess growths of an unknown compound. Gas chromatography–mass spectrometry confirmed that the growths are adipocere. The results herein reveal that adipocere can indeed form on defleshed bones under the right conditions and that even residual adipose and lipids in defleshed bones are sufficient to produce adipocere growth on the surfaces of bone.

KEYWORDS: forensic science, geoforensics, decomposition, burial, defleshed bone, fatty acids, saponification

At the completion of a recent experimental diagenesis study investigating the uptake and stability of trace elements in bone, a white-gray substance was found adhering to the surface of the bone samples. This substance had the appearance and texture of adipocere, and it was suspected that this was the substance's identity. The current study investigates the origin of adipocere and identity of the experimentally produced substance.

Adipocere (adipo = fat, cere = wax) is a grayish white, soft, soap-like, or waxy substance sometimes found on the corpses of animals and humans. Adipocere forms from the conversion of fats into insoluble, resistant lipid residues (1–5). Adipocere formation begins with the hydrolytic decomposition of fats into fatty acids (FA) (unsaturated and saturated) (Fig. 1), and the subsequent conversion of unsaturated FA into saturated FA and their 10-hydroxy (OHFA), 10-oxo fatty acids (OXOFA), and soap (2–4). Cleavage of FA groups from the basal glycerol unit is a hydrolysis reaction requiring water (Fig. 1). Hydrolysis can occur abiotically but occurs much more quickly and efficiently via bacterial enzymatic action. Formation of OHFA and OXOFA have been shown to be important in the formation of adipocere (2,5).

Experimental chemical observations of adipocere formation suggest that formation initiates within hours postmortem (6). However, visible adipocere accumulation occurs in as little as 6 weeks (4,7,8). Adipocere formation will continue as long as FA are available and acceptable conditions persist. Adipocere is incredibly resistant to decomposition and destruction by the environment and has been found on corpses as old as 7000 years (9). Resistance to decay arises from a requirement for large amounts of oxygen to effectively decompose adipocere (1,9). As a result, any corpse (or portion thereof) that becomes encased in adipocere is protected from decay as well. Adipocere encasements are virtually waterproof, air tight, and thermally insulating, thereby protecting interred remains from groundwater fluctuations (and dissolved ions), temperature fluctuations, microbial attack, and other destructive taphonomic factors.

Adipocere Formation

Free FA originating from the hydrolysis of adipose tissues react with ammonia produced during decay of organics (abiotic and biotic) to form soluble ammonium salts. Ammonium salts have an affinity for alkali metals, particularly Na, K, and Ca, and to a lesser degree, Mg (3,5,10,11). As ammonium salts attract and react with these metals, they form insoluble alkali salts or soaps that form resistant, relatively insoluble residues (1,10). Sodium ions from the interstitial fluid will react first to form sodium salts. Later, potassium ions from the cells or ambient water will replace sodium to form potassium salts (9). Higher abundance of sodium in the adipocere will result in a hard, crumbly soap, whereas greater abundance of potassium will result in "soft" soap with a paste-like texture (3). As the adipocere formation continues, sodium and potassium ions may be displaced by calcium or magnesium ions from the depositional environment (3).

The primary FA found in adipocere are the saturated FA, myristic, palmitic, and stearic acids (Fig. 2). However, unsaturated FA

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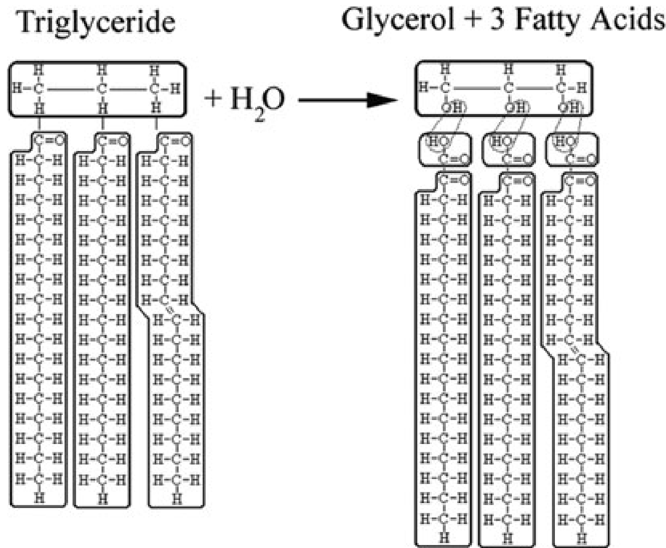

FIG. 1—*Hydrolysis reaction of a triglyceride molecule into one glycerol unit and three fatty acid units.*

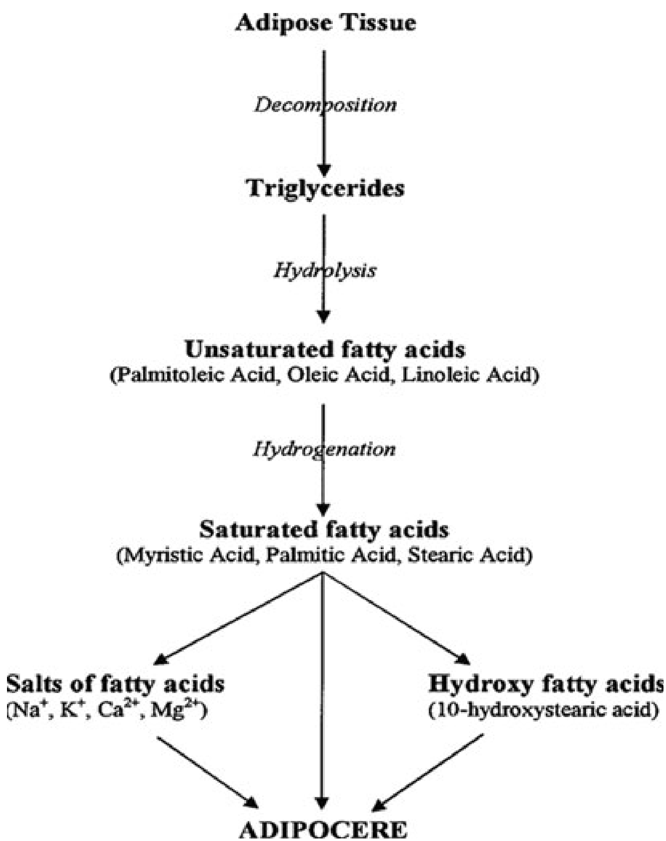

FIG. 2—*Decomposition of adipose tissues and formation of adipocere. From Forbes et al. (12).*

(oleic), salts of FA, OHFA, and OXOFA are also important (3,5). Most FA are liquid or semiliquid at body temperatures. Hydrogenation of FA to OHFA and later dehydrogenation of the OHFA to OXOFA result in elevation of melting points. Elevated melting points of these compounds lead to solidification and stabilization of adipocere and insoluble soaps (1,5).

TABLE 1—*Summary of conditions of adipocere formation. Compiled from Forbes et al. (3).*

Environmental Variable	Supported	Inhibited
Oxygen content	Anaerobic	Aerobic
Moisture	Dry-saturated soil	Desiccated tissues
pH	5.0–9.0 (~8.5 optimum)	pH < 5.0, pH > 9
Temp	22–40°C	<4°C, >40°C

Conditions

Although originally thought to require waterlogged, anaerobic conditions, adipocere formation is now known to occur in a variety of burial environments (1,3,9). Illuminating experimental studies of adipocere formation were conducted by Forbes et al. (3,12). They identified a variety of burial environments that supported and even promoted adipocere formation (summarized in Table 1). In addition, they identified specific burial environments in which adipocere formation was reduced or inhibited.

Forbes et al. (3) found that although moist and saturated soils promoted adipocere formation, dry soils too could support adipocere formation. Saturated conditions produced large masses of adipocere and offensive odors. Dry conditions also produced a large mass of grayish white adipocere but with no accompanying offensive odor. Because adipocere was shown to form in dry soil conditions, water within the tissues of the buried corpse must be sufficient to hydrolyze fat. However, adipocere formation would be improbable if the corpse was desiccated on the surface prior to burial. Anaerobic conditions were found to be most favorable as aerobic conditions produced no adipocere and the complete decomposition of the interred tissues (3). Forbes et al. (3) showed that acid soils produced no obvious adipocere formation (although trace adipocere was identified using gas chromatography–mass spectrometry [GC-MS]), whereas mildly basic soils (pH *c.* 8.5) produced abundant adipocere. Highly alkaline (or lime covered) conditions produced no adipocere. Forbes et al. (3) also found that excessively warm conditions (>40°C) inhibited adipocere growth, as did temperatures below 4°C. Thus, the optimum range of temperature for adipocere formation in their experiments was between 20°C and 37°C. Therefore, warm, moist, mildly alkaline, anaerobic conditions are considered optimum for adipocere formation. These conditions are also the optimal conditions for anaerobic bacterial growth, and any conditions that inhibit bacterial growth will also inhibit adipocere formation (including antibiotics, heavy metals, and other inhibitors).

In addition to the presence of water, bacteria have been shown to be necessary for development of adipocere. Early work had suggested that microorganisms may play an important role, but a series of studies published by Japanese scientists, particularly Takatori, cemented their role. Takatori and others found that bacteria are the primary agents of fat hydrolysis in the burial environment and necessary for the conversion of FA to OHFA and OXOFA (2,5). They found that bacteria such as *Pseudomonas* sp., *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Clostridium perfringens* could produce 10-hydroxy stearic acid from oleic acid. Only *M. luteus* produced OXOFA (under aerobic conditions). Because aerobic conditions have been shown to prevent adipocere formation, adipocere likely develops from the enzymatic activities of anaerobic bacteria, probably *Clostridium* sp. (3).

Environmental Interpretations

Studies into adipocere formation have indicated links between specific environmental conditions or stage of adipocere development and the "type" of adipocere formation. Forbes et al. (4) found

TABLE 2—*Relationships between burial conditions and fatty acids (FA). Compiled from Forbes et al. (4).*

Conditions	Relative Concentration FA
Wet environment	Higher palmitic (~78%), lower stearic (~8%), OHFA
Dry environment	Lower palmitic (~53%), higher stearic (~30%)
Early-stage or inhibited adipocere formation	High oleic (>20%)
Later-stage adipocere formation	Higher palmitic, lower stearic

OHFA, 10-hydroxy fatty acids.

that the FA composition of most samples was relatively consistent (palmitic acid being the major component) despite different burial conditions. However, different adipocere types could be determined by the relative percentages of the primary FA and were related to certain burial conditions (Tables 2 and 3). For example, wet environments produce adipocere with higher relative abundance of palmitic acid and lower stearic acid. Palmitic versus stearic compositions of samples from dry environments are more evenly distributed. In addition, wet environments were the only environment of those tested that was shown to produce OHFA (4). High oleic acid concentrations suggest early-stage adipocere formation, whereas high palmitic, low stearic acid compositions suggest later-stage formation (Table 2). Furthermore, increasing myristic acid composition appears to correlate with warmer, saturated, and mildly alkaline (more favorable) conditions (Table 3).

Methods

Experimental Material

Recent bovine (*Bos taurus*) femora from recently slaughtered animals from local slaughterhouses were used in this study. The femora were frozen immediately after animal slaughter to prevent premature decay and to aid in sample preparation. Later, 25-mm-long transverse sections were cut from the femoral shaft using a band saw (Fig. 3). Marrow within the femoral sections was removed, as well as excess flesh (muscle) and periosteum (when possible). Bone sections, or "disks," were then processed into three test groups. Treatment of these groups follows below.

Experimental Reactors

Individual sample chambers were necessary to maintain controlled conditions. Therefore, 32-oz. sealable plastic sample jars, or

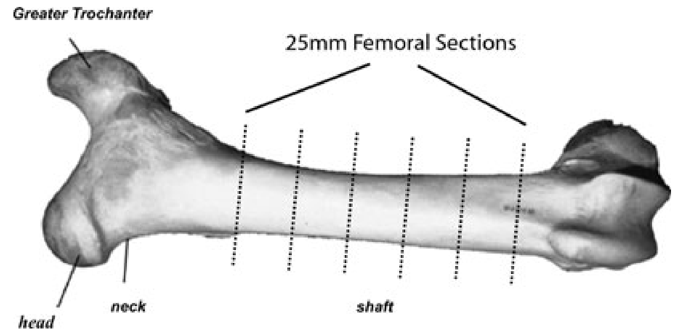

FIG. 3—*Schematic of bovine femur with femoral sections (bone disks) indicated.*

"reactors," were assembled for each individual sample specimen creating a diagenetic microcosm (Fig. 4). Plastic sample jars (reactors) were chosen instead of glass because of funding constraints. Each reactor contained 900 g of artificial sediment, 300 mL of buffering solution (Table 4) and had specified combinations of pH, bone sample type, and microbial content.

Artificial soil was utilized in this study to reduce the heterogeneity of naturally occurring sediments and soils and to eliminate the difficulties in attempting to homogenize them. This artificial soil was composed of 95% (by mass) fine- to very fine-grained (0.1–0.25 mm) quartz sand. The remaining 5% of the soil was composed of white kaolin clay, and 0.50 g per reactor of minced straw. Straw was added to provide a plant organic component to the soil. Soil constituents were mixed in a roller mill for 8 h to homogenize. Artificial soil (900 g) was weighed and placed into each sample reactor (Table 5). Bone disks were buried midway in the artificial soil (Fig. 4). Reactors were flooded with 300 mL of buffering solution (Table 4), and the soil remained saturated for the duration of the experiment.

Maintaining Eh-pH

Eh-pH conditions are two of the most important factors in bone modification and therefore must be addressed in any research investigating taphonomy. Eh, or oxidation/reduction potential, in a small closed system is very difficult to control and often tends toward negative values (anoxic) in the immediate areas around buried bones, despite external environmental conditions (13,14). Therefore, oxygen levels (Eh) within the reactors were allowed to progress naturally, and no attempts were made to control the values. Anoxia

TABLE 3—*Relative percent composition of fatty acids (FA) of tissue in various conditions. A characteristic FA composition of the original adipose tissue is included for comparative purposes.*

	Saturated FA				Unsaturated FA	
	Myristic ($C_{14:0}$)	Palmitic ($C_{16:0}$)	Stearic ($C_{18:0}$)	10-Hydroxy Stearic	Palmitoleic ($C_{16:1}$)	Oleic ($C_{18:1}$)
Adipose	1.4	32.4	25.8	0.0	0.9	36.0
Control	5.1	58.7	29.2	1.1	0.0	6.0
Alkaline	3.3	60.8	29.1	0.0	0.0	6.9
Acidic	2.0	48.8	29.7	1.9	0.3	17.3
Lime	3.2	35.3	9.7	0.0	4.8	45.6
Warm	7.4	55.5	31.0	0.3	0.0	5.6
Cold	1.4	27.8	16.3	38.8	0.5	15.2
Dry	1.2	58.9	35.0	0.0	0.0	5.1
Saturated	4.2	65.2	27.5	0.7	0.0	2.5

Alkaline = moderately alkaline burial environment; Acidic = highly acidic burial environment; Lime = burial with lime; Warm = burial environment at 40°C; Cold = burial environment at 4°C; Dry = dry burial environment, soil dehydrated; Saturated = wet burial environment, soil saturated. From Forbes et al. (3).

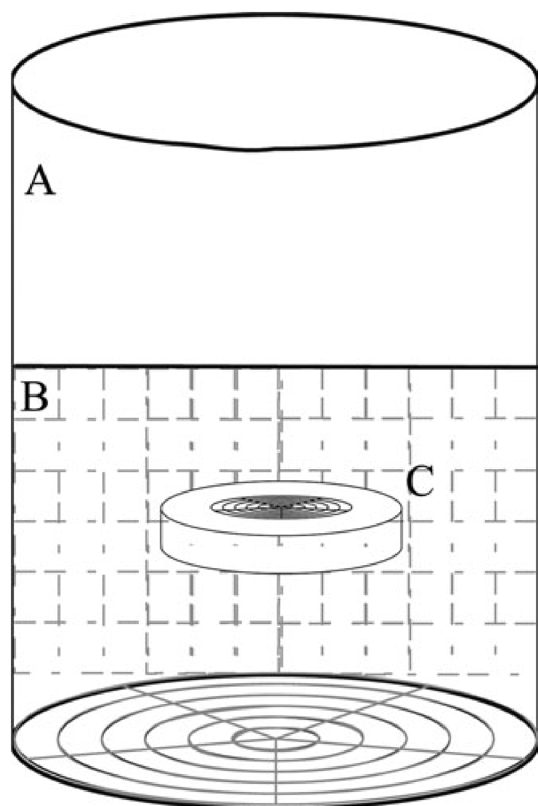

FIG. 4—*Diagrammatic representation of a sample reactor; (A) buffering solution; (B) artificial soil; and (C) bone sample.*

TABLE 4—*Buffer solutions and corresponding pH ranges used in reactors.*

Buffer A	0.1 M potassium hydrogen phthalate + 0.1 M HCl pH 3.5–4.0
Buffer B	0.1 M potassium hydrogen phthalate + 0.1 M NaOH pH 4.0–6.0
Buffer C	0.1 M KH_2PO_4 + 0.1 M NaOH pH 6.0–8.0
Buffer D	0.025 M $Na_2B_4O_7$ (borax) + 0.1 M HCl pH 8.0–9.0

TABLE 5—*Components of artificial soil in each "reactor."*

Component	Mass (g)
Quartz sand	854.50
Kaolin	45.00
Straw	0.50
Total	900

within the reactors was expected. However, Eh values were monitored throughout the duration of the experiment.

Normally, pH can be easily controlled via buffered solutions (Table 4). The pH values from 3.5 to 9.0 were divided into "cells" with a range of 0.5, such that 11 cells with 0.5 pH range were available. Reactors within the major test groups were assigned one of these starting pH values (no replicates), and attempts were made to maintain the established ranges by the maintenance of buffered solutions in each reactor. The pH of the solutions was monitored closely over the duration of the experiment, and adjustments were made accordingly (at least initially). Shortly into the experiment, it became abundantly clear that the buffer solutions and manual attempts at pH modifications were incapable of controlling the pH of the reactors. Dissolution of bone (hydroxyapatite) from the relatively large femoral sections and build-up of microbially produced basic compounds (i.e., ammonia) coupled with a rather small reactor vessel volume are the likely cause of the failure of the buffer solutions. pH conditions were monitored using a Mannix DPH8601K digital pH meter with dual-function, gel-filled electrode, three-point calibration, and automatic temperature correction (General Tools, NY, NY).

Experimental Groups

Three primary test groups were included in this study in an attempt to characterize various taphonomic factors during the early-fossilization process, under controlled, experimental laboratory conditions. Environmental, biological, chemical, and material factors contribute to the complexity of this period of time, and although all variables cannot be addressed, some parameters can be addressed. The three primary test groups are outlined below.

Mineral (X) Series—X-series samples were desiccated and stripped of as much organic matter as possible. Preparation generally followed Linden et al. (15), Tucker (16), and Zazzo et al. (17). Ethanol, acetone, hydrogen peroxide (3% H_2O_2), and sodium hypochlorite (6% NaOCl) were used to strip the collagen, other proteins, and lipids from the bone matrix. Eleven X-series bone samples were placed into reactors and flooded with solutions.

Sterile (S) Series—Attempts were made to prevent microbial activity in the S-series sample reactors. Sterilization would have removed any effects that microbial activity may have on burial environment. S-series samples remained frozen after the initial sample cutting until reactors and reactor solutions were prepared. In addition, the reactors of this test group were poisoned with 50 mL of mercurous chloride (Hg_2Cl_2) solution (0.02 g/mL) in an attempt to kill microbes and prevent their future growth following the experimental design outlined by Zazzo et al. (17). However, attempts to maintain sterility failed (see Results).

Microbe (M) Series—M-series reactors tested what role, if any, microbes (bacteria and fungi) have in the burial environment. A microbial solution (3.0 mL) extracted from common, natural environments was utilized to inoculate the reactors of this group. Microbes were obtained by sampling pond mud, horse manure, and decaying flesh. Minced straw already in the artificial soil acted as a microbial organic substrate, and 0.27 g of NH_4NO_3 (fertilizer) was added to provide nutrients for the microbes following Zazzo et al. (17).

Experimental Duration

After assembly, each reactor was sealed, stored in a dark room maintained at about 20°C, and agitated periodically over the duration of the experiment (18 months) (17). One should note that the storage temperature was about 10°C warmer than typical soil temperatures. However, the author felt that a stable, controlled temperature, even at 20°C, was justifiable for the sake of reducing variables. In an attempt to limit variables, possible contamination, and to maintain stable internal environments, reactors remained sealed for most of the experiment. As a result, few observations

regarding chemical changes were possible over the duration. The reactors were only briefly opened periodically to test the Eh/pH of the solutions (see Results). Berner (10) found that anaerobic conditions were easily maintained even after repeated opening of his experimental vessels.

Gas Chromatography–Mass Spectrometry

Methods for GC-MS generally follow Forbes et al. (4). Samples (*c.* 200 mg) of suspected adipocere were collected from deposits on the bone (Fig. 5) and carefully weighed in sterile vials. Chloroform (1 mL) was added to the vials and sonicated for 15 min. Vials were centrifuged, and the chloroform layers (middle) were drawn off and placed in new, sterile, snap-top vials. Hexamethyldisilazane (0.25 mL) was added, and the vials were heated at 70°C for 10 min. Vials were removed and refrigerated until testing. All analyses were performed on a Hewlett–Packard (Agilent, Technologies, Inc., Santa Clara, CA) 5890 Series II gas chromatograph coupled with a Hewlett–Packard 5970 Series mass selective detector. A 1-μL aliquot of the sample was manually injected into a HP5-MS-fused silica capillary column (30 m × 0.25 mm × 25 mm, 5% phenylmethyl polysiloxane). Helium was used as the carrier gas at a flow rate of 20 mL/min. The column temperature was heated to 100°C for 1 min and increased at 7°C/min to 275°C, and held for 5 min. The ionization voltage was 70 eV, and the ionization current was 60 mA. Each sample was scanned for masses from 50 to 450 *m/z*. All spectra were collected using HP-ChemStation software (Agilent Technologies, Inc.). A 1-μL aliquot of chloroform was injected into the column between each sample and analyzed to ensure that the column was flushed of residual sample and to eliminate memory effects.

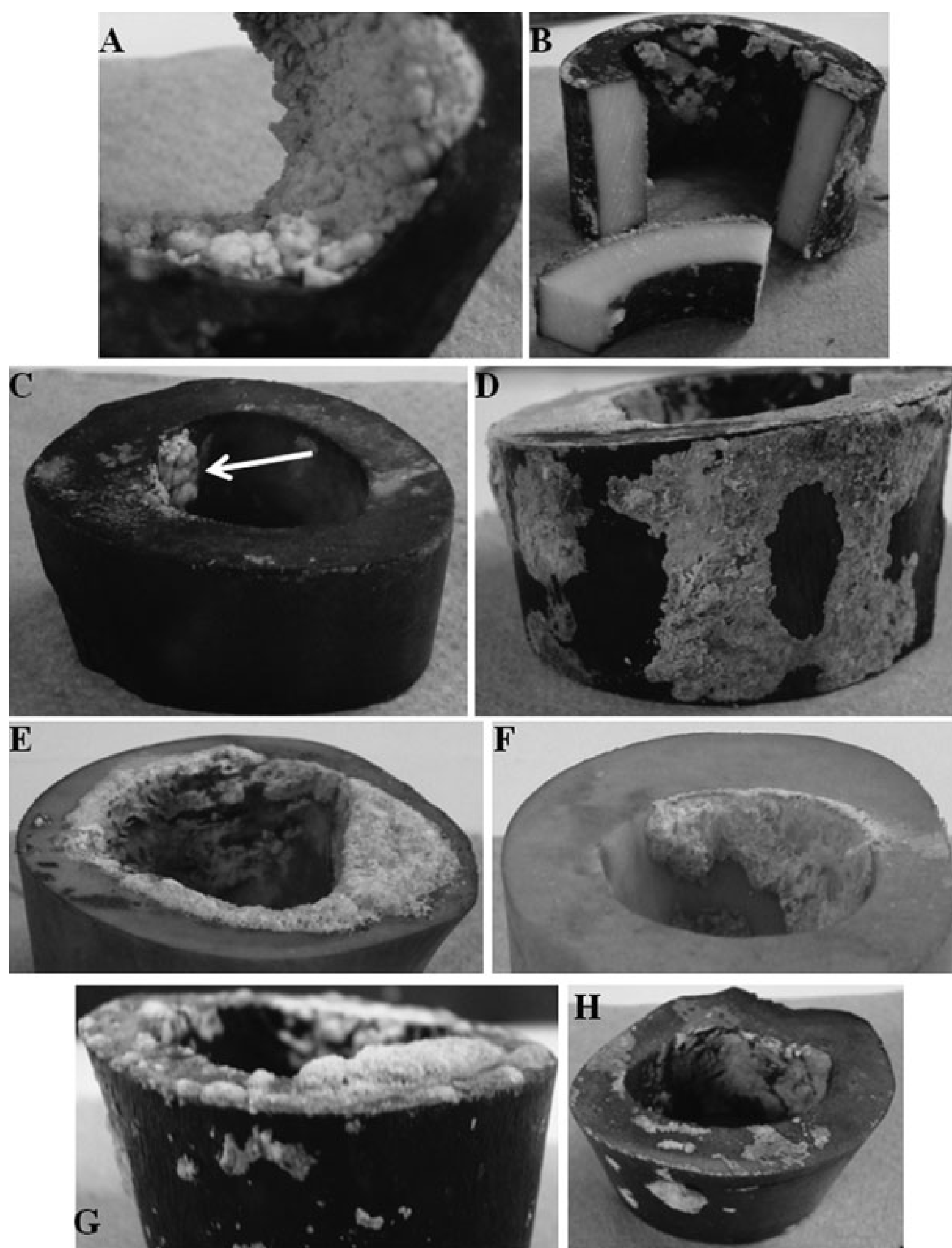

FIG. 5—*Bone samples exhibiting adipocere accumulations; (A) Sample Ls; (B) Sample Js; (C) Sample Mm2; (D) Sample Ks; (E) Sample Jm; (F) Sample Lm; (G) Sample Ns; and (F) Sample Is.*

Results and Discussion

Seven samples were found to possess strange encrustations at the completion of the experiment (Table 6, Fig. 5). GC-MS was utilized to confirm that the encrustations were adipocere following the methods of Forbes et al. (4) (see also Methods). Results indicate the requisite FA common to adipocere. On most samples, the adipocere growths were found to be grayish white, soft, and soapy or waxy (Table 6). A few other samples possess adipocere that was harder and crust-like. All samples exhibiting hard adipocere are derived from sample reactors, which utilized potassium hydrogen phthalate (KHP).

All adipocere encrusted samples were found on bone samples belonging to the S- and M-series, with final pH values averaging 9.12. High pH values in the reactors likely resulted from solution buffering caused by dissolution of bone and build-up of basic compounds produced by microbial decay of proteins (ammonia), but may also have resulted from leaching (Na) from the plastic reactor vessels. None of the bone samples in the X-series developed adipocere growths. X-series samples were subjected to chemical treatments to strip the bone of as much organics (protein and lipids) as possible. Therefore, no residual adipose tissue was available for the conversion process. X-series samples represent bones that may have undergone anthropologic processes, such as cooking or maceration, or taphonomic processes such as weathering, oxidation, or scavenging.

Microbial decomposition converts oleic acid into palmitic acid. Forbes et al. (8) showed that oleic acid concentrations decrease as palmitic acid concentration increase such that samples with the highest relative percentage of palmitic acid also have the lowest relative percentage of oleic acid. They proposed that the stage of adipocere development (maturity) could be classified based on the relative FA composition. Early-stage adipocere (immature) exhibits high-relative oleic and low-relative palmitic acid concentrations. Late-stage (mature) adipocere exhibits low-relative oleic and higher-relative palmitic acid. Table 7 shows the relative composition and classifies the stage of development of the adipocere on each sample analyzed in this study. All samples were subjected to the same duration of burial, yet exhibit different stages of adipocere development. These results confirm Forbes et al.'s (8) assertion that the stage of adipocere formation cannot be attributed to the duration of burial.

As noted earlier, microbial activity is required for the development of adipocere. However, several confirmed adipocere samples belong to the S-series, which was intended to stay sterile for the duration of the experiment. Therefore, S-series samples should not have developed adipocere. Methods for preparation of this series followed Zazzo et al. (17). Regrettably, a most unfortunate typographical error in their study stated that Hg_2Cl_2 (mercurous chloride) was used as a poisoning agent to prevent microbial growth. However, mercuric chloride ($HgCl_2$) was used instead (Antoine Zazzo, personal communication). Mercuric chloride is more soluble in water than mercurous chloride, thus more toxic to microbes. As a result, the S-series reactors did not remain sterile and microbial growth occurred in this series as quickly as in the others. In an attempt to sterilize the reactors or at least inhibit microbial growth in them, reactors were treated using 6% reagent-grade NaOCl. Ultimately, microbial growth was not inhibited to any substantial degree.

TABLE 6—*Samples with suspected adipocere encrustations. First letter of the sample name indicates the bone from which the sample was taken. Second letter of sample name indicates the reactor series the sample belongs to. Numbers were used in the sample name when multiple samples in one reactor series originated from same bone.*

Sample	Final pH*	Buffer Solution[†]	Consistency of Adipocere
Is	9.02	KPO_4/NaOH	Waxy
Js	9.10	KHP/NaOH	Hard soapy
Ks	8.83	Borax/HCl	Waxy
Ls	9.28	NaOH/KHP	Hard
Ns	9.12	KHP/NaOH	Hard
Jm	9.59	KHP/NaOH	Hard soapy
Km	9.12	NaOH/KPO_4	Waxy
Lm	9.09	KPO_4/NaOH	Soft
Mm2	8.97	KHP/HCl	Hard

*pH reading at completion of experiment.
[†]Largest volume buffer notated first.

Conclusions

Prior to this study, published research has reported or assumed that abundant adipose tissue was necessary for adipocere formation. No indications that adipocere could form on defleshed bones with little fat available were made. The results described above reveal that adipocere can indeed form on defleshed bones under favorable conditions, indicating that even residual adipose tissue and other lipids found in defleshed bones are sufficient to produce adipocere growth on the surface of buried bone. Furthermore, these results confirm prior studies indicating that the stage of adipocere formation cannot be attributed to the duration of burial. Oleic acid concentrations decrease as palmitic acid concentration increase such that samples with the highest relative percentage of palmitic acid also have the lowest relative percentage oleic acid.

Future analyses to determine the alkali metal composition of the adipocere will help classify the stage of development for these samples. As stated earlier, early-stage adipocere is high in Na and K. These metals are progressively replaced by Ca and Mg (to a lesser extent) as the adipocere matures.

TABLE 7—*Relative fatty acid (%) composition and stage of formation of confirmed adipocere samples.*

Sample	Myristic Acid	Palmitic Acid	Oleic Acid	Stearic Acid	Stage of Formation*
Is	2.0	30.7	41.7	25.5	Early
Ls	4.1	51.8	32.4	11.8	Early–intermediate
Mm2	3.3	59.0	17.8	20.0	Intermediate
Ns	7.5	64.6	19.8	8.1	Intermediate
Km	5.6	68.8	8.0	17.5	Late
Jm	3.2	70.5	8.1	18.3	Late
Ks	3.8	77.9	2.9	15.4	Late

Sample name corresponds to bone sample.
*Based on Forbes et al.'s (8) stage classification.

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