S. Pfeiffer,[1] *Ph.D.; S. Milne,*[1] *B.Sc.; and R. M. Stevenson,*[2] *Ph.D.*

The Natural Decomposition of Adipocere*

REFERENCE: Pfeiffer S, Milne S, Stevenson RM. The natural decomposition of adipocere. J Forensic Sci 1998;43(2):368–370.

ABSTRACT: Adipocere is a waxy substance which sometimes forms from the adipose tissue of dead bodies, especially when they are under water. A disinterment in southern Ontario lead to the recovery of extensive adipocere from an interment which occurred in AD 1869. Subsequent laboratory research was designed to explore the conditions under which adipocere will disappear, the goal being to identify strategies for estimating a range of time since death in cases where adipocere is present. Varieties of aerobic or facultatively anaerobic microorganisms from the surface of the adipocere were separated and identified. In culture, the gram positive bacteria were able to degrade the adipocere. We propose that the persistence of adipocere is related to the exclusion of gram positive bacteria from the burial environment. 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.

KEYWORDS: forensic science, forensic pathology, adipocere, grave wax, time since death, inhumation in water, lipolytic bacteria

Adipocere is a waxy substance that is associated with dead bodies that have been under water, in moist soil or in other moist environments for prolonged periods of time. Formed from adipose tissue in the presence of water and bacteria (1,2), it is a stable compound that has been associated with the mummification of the body (3). Adipocere is composed of hydroxy fatty acids, predominantly 10 hydroxy stearic acid. Different species have characteristic adipose tissue fatty acid patterns. In the human,the most common unsaturated fatty acids are oleic acid (18:1), linoleic acid (18:2) and palmitoleic acid (16:1). The saturated fatty acids found in highest proportion are palmitic acid (16:0) and stearic acid (18:0). For adipocere to form, water must be added across the unsaturated double bond of constituent fatty acids, forming the characteristic hydroxy fatty acids. Hydroxy stearic acid, formed by the addition of water across the unsaturated double bond of oleic acid, is the only fatty acid which has been proven to be a constituent of adipocere. Other hydroxy fatty acids may be involved in adipocere's formation, but to date, studies have only revealed conflicting information on this point (3–5). There are two forms of 10 hydroxy stearic acid which make up adipocere, *i*-hydroxy stearic acid and θ-hydroxy stearic acid (6). The stability of adipocere can be attributed to increased interactions between the added hydroxyl groups and the COOH terminal of the fatty acids. These covalent, ionic, hydrogen bonds and Van der Waals forces greatly increase the melting point and thus the stability of adipocere.

It is not clear how adipocere decomposes, nor how it interacts with other factors during the decomposition process (7,8). The process of decomposition involves both anabolic and catabolic processes resulting in the breakdown and reuse of most of the cellular molecules. It has been hypothesized that soil microbiota, including bacteria, fungi and algae (9) will affect decomposition of adipocere. Our focus will be on bacteria commonly found in the soil of the region from which the adipocere was recovered.

Material and Methods

The widening of a highway near Toronto, Ontario, necessitated the relocation of part of a "pioneer cemetery," a family plot used from 1844 to 1906. Only three burial sites were distributed: the double burial of two unidentified juveniles and a pair of identical double-sided yellow pine coffins lying in parallel, about 2 ft apart, belonging to a married couple identified by a shared headstone. At the time of disinterment, in September, 1991, both adult coffins were below the water table and had to be drained before excavation could proceed.

Both adults were natives of Ireland, each was 60 years old at death. He died in November, 1870, and she died two years prior, in January, 1869. Skeletally, both adults were short, the estimated stature of the male being 165 cm and the female being 150 cm. The asymmetrical development of the man's arm musculature with right side dominant and the healed fracture of the left thumb is consistent with his census classification of farm laborer. Her skeleton showed degenerative osteoarthritis at the hips, general osteopenia and almost edentulous jaws with no indication of accommodation to dentures (10).

Given the similar size, age and time of death of these two adults, their difference in preservation is noteworthy. While his skeleton was well preserved with no soft tissue remaining, her coffin held a massive block of adipocere, approximating a torso in its shape and surface features (breasts, buttocks) and a poorly preserved axial skeleton (Fig. 1). A thick, dark gray layer with the consistency of fine soap suds covered the adipocere. Analysis established that the "bloom" was comprised predominantly of *Aeromonas hydrophila*, a gram-negative facultatively anaerobic bacterium, common in water.

All excavators wore heavy disposable rubber gloves. The adipocere and adhering organic matter was wrapped in heavy plastic as it was removed from the coffin. Subsequent examination under standard laboratory conditions showed that the block of adipocere was odorless, creamy white in color and chalk-like in consistency.

[1]Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

[2]Department of Microbiology, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

*Components of this research were presented at the 1993 meetings of the American Academy of Forensic Sciences, Boston, and the 1996 meetings of the Canadian Society of Forensic Science, Hamilton, Ontario.

Received 17 Feb. 1997; and in revised form 21 May and 18 July 1997; accepted 28 July 1997.

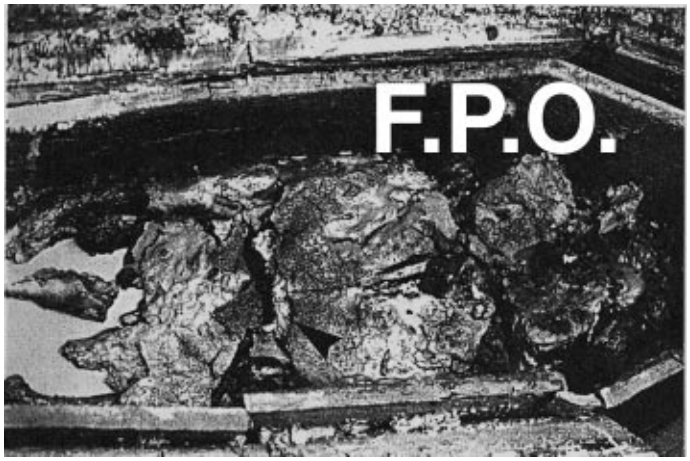

FIG. 1—*The opened coffin; The skull (not visible) was toward the narrowed end. The mass of organic material can be distinguished as breasts, abdomen and slumped buttocks, with a sharp break at the base of the abdomen (arrow) exposing the underlying whitish adipocere. Standing water covers the leg bones. Photo courtesy of Archaeological Services Inc, Toronto.*

Adipocere of similar color and consistency filled the medullary cavities of the long bones. The bone in direct contact with the adipocere was soft and exfoliating. Regions which were not in direct contact with the adipocere, such as the auricular surfaces of the hip bones, were in good condition. The bones were reinterred after analysis. The bulk of the adipocere was retained and frozen in a standard freezer unit, with a small sample retained at room temperature in an anaerobic environment in anticipation of research approaches which had not yet been designed. While both the frozen and unfrozen samples were isolated, they were not maintained in strictly sterile conditions. The research described here was begun in 1995.

Isolation of Microorganisms

Small samples from both the frozen and unfrozen adipocere were applied to Trypticase soy agar plates. Each plate was incubated under aerobic conditions at a temperature of 25°C. Colonies of 8 different morphologies were observed on the plates. Small amounts of adipocere from each sample were also put into a brain-heart infusion broth in test tubes and incubated at 25°C. After several days an aliquot of this was streaked onto a Trypticase soy agar plate and incubated at the same temperature. Only one kind of colony was observed on the plate. The nine different colonies were transferred using standard sterile plating techniques four times in order to obtain pure cultures.

Microbial Identification Tests

Standard primary identification tests were applied to characterize the bacterial cultures (11). All of the pure cultures were gram-stained in order to differentiate them into two general groups, gram positive or gram negative. The catalase test was performed in order to determine the presence of the enzyme catalase, present in most aerobic and facultatively anaerobic bacteria. The oxidase test was used to determine the presence of cytochrome c, a heme containing protein that acts as one of the electron carriers in respiration. Hugh-Leifson's OF medium was used to test whether glucose was metabolized, and whether the process was oxidative or fermentative processes. Colony morphology, pigments, cell shape and motility were examined and recorded using standard methods and terminology. These test results are sufficient to allow the bacterial isolates to be assigned to genera or groups of genera (11).

To complete bacterial identification conveniently and quickly, commercially available multi-test systems were used. The API 20E strip (bioMerieux Canada, Inc., St-Laurent, PQ) uses miniaturized, dehydrated versions of standard enteric tests for nitrate use, amino acid decarboxylation, urea formation, and various sugar fermentations. This test system was used for one gram negative culture which appeared to belong to the enterobacteria family. Bio-log GN Microplates (Bio-log Inc., Hayward, CA) assay bacterial activities on 95 carbon source substrates by using a tetrazolium dye indicator. Three gram negative cultures were analyzed using these plates.

Degradative Activities

Lipolysis was determined on Tween 80 agar media, containing 1% polyoxyethelene sorbitan monooleate, an 18 carbon fatty acid. Oleic acid, also an 18 carbon fatty acid, is the fatty acid hydrolyzed in the formation of adipocere. Lipolytic bacteria produce a halo effect in the agar as free fatty acids precipitate as a calcium salt.

For those samples where lipolysis was observed on Tween 80, a direct test of the ability of bacteria to breakdown adipocere was undertaken. An adipocere suspension was made up by emulsifying 0.521 g of adipocere in 5 mL of sterile saline solution. Five microliters of this suspension was then mixed with 3 mL of agar solution and poured as a thin overlay onto a standard TSA plate. (Small adipocere particles could be seen within the overlay). Bacteria were then plated on to these plates and incubated in order to visually assess their ability to breakdown adipocere.

Results and Discussion

The bacteria isolated from the surfaces of two samples of adipocere include seven genera, those being the gram-negative *Pseudomonas, Serratia, Alcaligenes* and *Enterobacter* and the gram-positive *Bacillus, Nocardia* and *Cellulomonas* (Table 1). *Bacillus* alone was isolated from the sample stored unfrozen; the other isolates were all from the surface of the frozen sample of adipocere. All of the bacteria identified are common soil microorganisms; *Pseudomonas* and *Serratia* are commonly found in water. When plated on nutrient agar plates with an adipocere overlay, the three gram-positive isolates hydrolyzed the adipocere. Lipolysis on Tween 80 agar was also observed. Among the gram-negative bacteria, *Serratia marcescens* exhibited lipolytic ability on Tween 80 agar.

The presence of adipocere in the case described here is not associated with the mummification of any soft tissue. Indeed, the decomposition of the bone seems to have been speeded by the presence of the adipocere, perhaps because it hindered water drainage. The size and shape of the torso adipocere, combined with the

TABLE 1—*Results of the screening of adipocere surfaces for bacteria.*

Genus, Species	Lipolysis	Gram Reaction
Pseudomonas acidovorans	no	negative
Pseudomonas stutzeri	no	negative
Enterobacter sp	no	negative
Serratia marcescens	yes	negative
Alcaligenes latus	no	negative
Bacillus sp	yes	positive
Cellulomonas sp	yes	positive
Nocardia sp	yes	positive

osteoarthritic changes to the hip joints, suggest that the female buried in 1869 was obese and that this super-abundance of adipose tissue combined with burial below the water table encouraged adipocere formation. The absence of adipocere in the male beside her could be explained by a lean physique, patterns of subsoil water flow, or other unidentified factors.

The bacteria isolated from the surfaces of the samples of adipocere belong to genera which might be expected in Ontario soils. While the samples of adipocere were not handled sterilely from the time of their exposure, they were removed and stored using methods that avoided subsequent contact with possible sources of airborne contamination. Thus the most parsimonious explanation is that the microbes originated from the original source of the material. Bacteria appeared to occur only on the exposed surfaces of the samples, rather than being intermixed with the adipocere. This would be consistent with colonization of an available surface with autochthonous soil and water microbes. Because a bloom of *Aeromonas* was overlaying the adipocere at excavation, we anticipated that this microbe would be present within the samples and might be demonstrated to be using the adipocere as a source of energy. This was not the case.

Two of the species identified, *Pseudomonas acidovorans* and *Pseudomonas stutzeri*, were identified by Takatori et al. (2) as possibly involved in the transformation of adipose tissue to adipocere under aerobic conditions. This observation suggests that some of the gram-negative bacteria isolated from the adipocere sample could be the bacteria responsible for adipocere formation. In order for the bacteria to hydrolyze fatty acids they must first be present in the cadaveric environment. This case shows extensive formation of adipocere within the medullary cavities of the intact long bones. Access to the inner cavities of long bones may be somewhat restricted by the surrounding cortical bone. Therefore, we tentatively favor the hypothesis that the formation of adipocere occurs through chemical interaction with water in a slightly anaerobic environment, but this case does not offer decisive support for either hypothesis.

All the gram-positive bacteria found within the adipocere were capable of lipolyzing the adipocere, and hence contributing to its decomposition. Most gram-negative strains tested showed no lipolytic activity in the presence of adipocere. Most of the isolates identified in this study, including the exuberant growth of *Aeromonas*, are gram-negative. We postulate that at the time of disinterment they were simply using the adipocere as a surface upon which to colonize while using nutrients in the surrounding soil. Why had the adipocere persisted for over 120 years? The mass initially formed was very large, so considerable degradative activity would be needed. The presence of a large volume of gram-negative organisms may have acted to exclude gram-positive organisms and hence to discourage adipocere degradation. Microbial degradation occurs at the surface of the material, or internally where the microbes penetrate (e.g., filaments of bacteria, fungi). The formation of adipocere will therefore be a function of the presence of adipose tissue, abundant water, a slightly anaerobic environment, and possibly gram-negative bacteria. Its subsequent disappearance will be a function of the presence of gram-positive bacteria and an aerobic environment; the rate of disappearance will be affected by the mass of the material formed.

Acknowledgments

We thank Archaeological Services Inc., Toronto, Ontario, for the opportunity of working on this site. Excavation, analysis and photography was done with the assistance of J. Christopher Dudar and Elizabeth Smoczynski. Analysis of the *Aeromonas* was done by Prof. J. MacInnes, Department of Pathobiology, University of Guelph.

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Additional information and reprint requests:
Susan Pfeiffer, Ph.D.
Department of Human Biology & Nutritional Sciences
University of Guelph
Guelph, Ontario, Canada N1G 2W1