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Adipocere withstands 1600 years of fluctuating groundwater levels in soil

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ABSTRACT

An extraordinarily well-preserved skeleton of a child, interred in a stone sarcophagus in the Late-Roman era, was discovered in the city of Mainz (Germany) in 1998, covered with a puff pastry-like substance assumed to be adipocere. It is the first time that this substance, which is derived from fat under oxygen-deficient conditions and prevents corpses from decaying, has been discovered on corpses buried under conditions described in the present paper.

The body was buried at groundwater level (2.9 m below surface) in a moist zone close to the Rhine that was affected by seasonally fluctuating groundwater levels. The fluctuating groundwater levels would appear to have had an effect on the degradation of the interred body. The discovery of the skeleton gave us an excellent opportunity to examine fatty acid material which had been subjected to prolonged fluctuating aerobic and anaerobic conditions in a moderate environment. The body's fatty acid composition and ^{13}C abundance were determined and compared with modern adipocere values. Element analysis of the stone sarcophagus in which the child was buried provided information on the burial environment. Our findings indicate that fatty material must have been converted into adipocere under anaerobic conditions in periods of high water levels, leaving the material open to decay during periods of low water levels. The fact that the excavated body was still covered with adipocere 1600 years after the burial clearly shows the robustness of the material against decay.

1. Introduction

In 1998, the General Department of Cultural Heritage Rhineland-Palatinate, Department Archaeology in Mainz carried out excavations in Mainz and discovered several graves, one of which contained an extraordinarily well-preserved skeleton of a small child (64 cm tall, 6–12 months old; Zipp, 2003) (Fig. 1). The child was most likely buried in the 4th century, an assumption based on the discovery of a ceramic jug in close vicinity to the sarcophagus (Witteyer, unpublished). The child's bones were surprisingly well-preserved; they were neither particularly porous nor fragmented. In addition, they (in particular the arm and leg bones) were covered with a hard, cement-like crust that resembled layers of puff pastry, which was assumed to be adipocere.

It is believed that this crust contributed to the excellent preservation of the child's body (Fig. 2 a and b). As far back as 1786, Fourcroy and Thouret (Fourcroy, 1789) were the first to describe the waxy remains of Parisian children. Adipocere, as they called it (adipo = fat; cere = wax), is a special type of post-mortem degradation in which neutral fat triglycerides are hydrolyzed into fatty acids and converted into insoluble hydroxy and saturated fatty acids e.g., stearic and palmitic acid (Gotouda et al., 1988). Adipocere is almost totally resistant to decay, which means that bodies covered in adipocere are protected from decay (Janaway, 1987; Micozzi, 1991; O'Brian and Kuehner, 2007; Froentjes, 1965; Ruttan and Marshall, 1917; Strassmann and Fantl, 1926). Adipocere develops from anaerobic bacteria (e.g., *Clostridium frigidicarnis*, *Clostridium perfringens*) and only forms when a body is buried in oxygen-deficient surroundings, i.e. moist areas (Vass, 1992; O'Brian and Kuehner, 2007; Bereuter et al., 1997; Bereuter, 2002). It is one of the most frequent types of post-mortem changes in corpses found in water (Kahana et al., 1999), glacial and permafrost areas (Murphy et al., 2003; Morgan et al., 1983) or in corpses wrapped in hermetic bags (Rothschild et al., 1996). In the case of the Tyrolean iceman, the combination of freezing, drying and conversion into adipocere most likely explains the excellent preservation of the body (Murphy et al., 2003; Bereuter et al., 1997; Bereuter, 2002).

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Fig. 1. View on the sarcophagus (external size $120 \times 62 \times 45$ cm) (Photo: Archäologische Denkmalpflege Mainz).

Although adipocere is derived from fat, it does not resemble fat. Children usually have greater proportions of fat on their arms and legs, something that immediately suggested adipocere as a major reason for the excellent preservation of the child's body. According to Wieners (1938) and Harcken (1952), newborn babies are particularly prone to adipocere formation. Based on babies' relatively high proportion of fat, this seems plausible (Dix and Graham, 2000). Since the formation of adipocere had previously not been described for late antiquity corpses interred in a sarcophagus, our initial interpretation was somewhat audacious. The present study therefore sets out to clarify whether the material was indeed adipocere. This involved determining (1) the body's fatty acid composition and ^{13}C abundance (2) and comparing them with modern adipocere values. Since the environment has a great impact on adipocere formation, the element spectra of the stone sarcophagus in which the child was buried were also analyzed in order to obtain detailed information on the burial environment.

2. Material and methods

2.1. Environment

The child, interred in a stone sarcophagus (external size $120 \times 62 \times 45$ cm, Fig. 1) was discovered at a burial site located near the northern arterial road of Mogontiacum (Latin name for Mainz). The gravesite is located in a moist area close to the banks of the Rhine. Efforts to drain the area had been ongoing since the 1st century (Witteyer, 2005). Nevertheless, historical records repeatedly mention fluctuating groundwater levels, with high groundwater levels occurring during autumn. The sarcophagus was embedded in fine, gray Rhine sand, which suggests that the groundwater level was low at the time of the child's burial. The sarcophagus did not contain any soil; it is possible that it filled with water during high groundwater levels, thereby creating an anaerobic environment. Pieces of cloth were also found on the body.

2.2. Sampling and sample preparation

Tissue samples (3 sub-samples of 5 g) were taken from the child's thorax and pelvis. These samples were compared to modern adipocere taken from the abdomen of a dead 50-year-old male who had been interred for 27 years in a modern cemetery (southwest Germany). This cemetery is located on clay soil whose tendency to retain water impedes post-mortem degradation. The exhumed corpse was covered with adipocere (gray-white, waxy substance, the thickness of the lipid armour ranged between 3 and 4 cm) and hence well-preserved. Only part of the peripheral extremities and the cranium showed signs of skeletalization. The bones were very elastic due to demineralization and the presence of residual amounts of collagen.

The tissue specimens were dried at 40 °C (48 h), ground and homogenized.

Stone samples (approximately 1×2 cm) were collected from the floor (i.e. the contact surface with the corpse) and lid of the sarcophagus.

2.3. Fatty acid composition

Prior to analysis, the specimens were pretreated with GC-FID (6890N, Agilent Technologies, Santa Clara, CA, USA). The fatty acid methyl esters were produced according to ISO-guide EN ISO 5509-2000 and EN ISO 15304-2002: 100 mg of the dry specimens were extracted with 1 ml of tertiary butylmethylether (TBME) to obtain the fatty proportion of the material. The specimens were then elutriated with 2 ml water and acidified with 0.5 ml hydrochloric acid, 2% HCl and extracted for the second time with TBME to educe the free fatty acids from the calcified crust. The extracted fatty acids were subsequently derivatized into fatty acid methyl esters (FAME) using trimethylsulfonium hydroxide (TMSH). The FAMEs were analyzed by GC-FID on a cyanopropyl column (CP-select CB for FAME; 100 m; ID: 0.25 mm; FT 0.20 μm) according to EN ISO 15304 and 5509 and compared to a standard mixture of fatty acid methyl

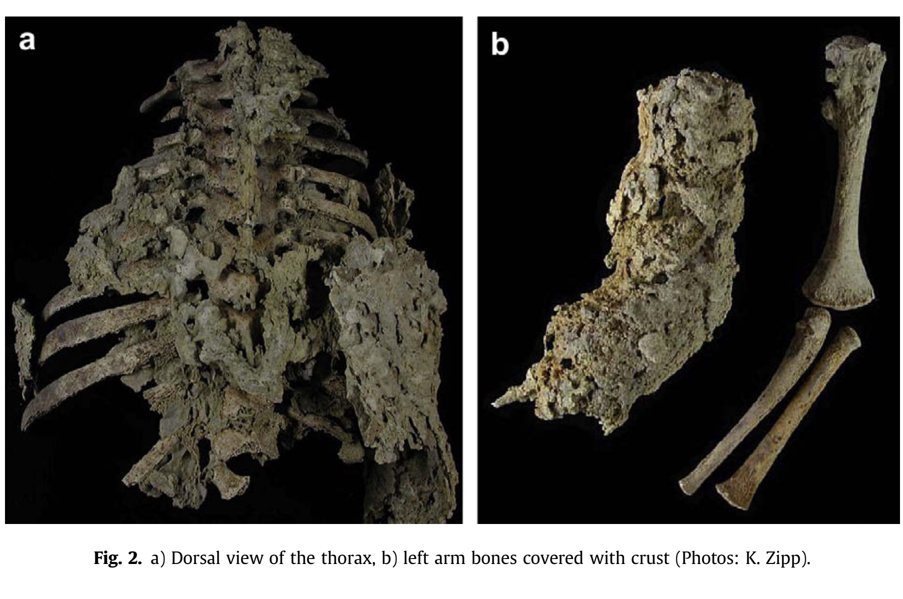

Fig. 2. a) Dorsal view of the thorax, b) left arm bones covered with crust (Photos: K. Zipp).

Table 1
Comparison of ancient and modern human adipocere.

Analysis	Roman child	Modern human specimen
Organic carbon ($g\ kg^{-1}$)	6.31 ± 0.5 thorax: 6.15 ± 0.37 pelvis: 6.47 ± 0.61	740.6 ± 4.9
C-14, C-16 and C-18 fatty acid methyl ester ratio	1:8:1.6	1:5.5:1.2
$\delta^{13}C$ (organic part) (‰ V-PDB)	Thorax: -25.59 ± 0.2 Pelvis: -25.57 ± 0.12	-26.80 ± 0.12
Inorganic substances	95 percent by weight calcite ($CaCO_3$), 1 percent by weight dolomite ($CaMg(CO_3)_2$) and 5 percent by weight quartz (SiO_2)	–

esters. The data were analyzed with the ChemStation software, version B.01.03, from Agilent Technologies.

2.4. Organic carbon (C_{org}) and ^{13}C abundance

Five aliquots of approximately 5 mg crust material (thorax and pelvis) were added to 10×10 mm Ag capsules. The successive addition of a total of 350 µl 2 N HCl led to the release of inorganic carbon. The samples were dried at 60 °C for 10 min in a Speedvac, packed in Sn foil and examined with an EA-IRMS system (Elementar Analysator: Euro EA, Eurovector, Milan, Italy; IRMS: MAT 253, Thermo-Finnigan, Bremen, Germany) for $\delta^{13}C$ values and C_{org} content. The measurement results were calibrated with measurements of the international NBS 19, CH 6 and USGS 40 standards. The modern adipose tissue specimens were analyzed with the same system. The $\delta^{13}C$ values of the samples were expressed according to the international V-PDB standard:

$$\delta^{13}C[‰V{-}PDB] = \left(\frac{R_{sample} - R_{V-PDB}}{R_{V-PDB}}\right) \times 1000$$

where $R = {}^{13}C/{}^{12}C$ and $R_{V\text{-}PDB} = 0.0111802$ (Werner and Brand, 2001).

2.5. X-ray diffraction

The mineral composition of the tissue specimens was analyzed with a Philips diffractometer PW 3710 (40 kV, 30 mA) with CuKα-radiation, equipped with a goniometer with a radius of 183 mm, a fixed divergence slit and a secondary graphite monochromator. The powdered sample was scanned with a step size of 0.02° 2 theta and a counting time of 3 s per step over a measuring range of 2–80° 2 theta. Starting from the qualitative XRD analysis, a quantitative analysis was performed applying Rietveld refinement using the AutoQuan® software (GE Inspection Technologies, SEIFERT Analytical X-ray, Germany). All results are given as the mean of five analyses.

2.6. Scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopy (EDX) analysis

The mineral composition of the sarcophagus (both from the sarcophagus floor and lid) was estimated by SEM (Leo 420, Leo, Oberkochen, Germany). EDX (Röntec EDWIN-NT, Röntec, Germany) was used to analyze the chemical elements that may have been present in the samples. The color of the sarcophagus material was determined on the basis of standard soil color charts.

3. Results

The child tissue specimens contained $6.31 \pm 0.5\ g\ kg^{-1}$ organic carbon; no significant differences were found between the samples taken from the thorax and pelvis (Table 1). The presumptive adipocere crust contained only traces of substances that are typical for adipocere: myristic acid methyl ester (36.4 min), palmitic acid methyl ester (40.1 min) and stearic acid methyl ester (43.4 min) (GC-FID chromatogram in Fig. 3). Other fatty acids were not detectable.

The C-14, C-16 and C-18 fatty acid methyl esters ratio of gray-whitish colored reference adipose material had a ratio of 1:5.5:1.2; the two child specimens had a fatty acid methyl ester ratio of 1:8:1.6(Fig. 4). The $\delta^{13}C$ values of the organic specimens of the modern adipocere compared were almost identical (Table 1). The child's $\delta^{13}C$ values of −25.6‰ (and slightly lower) were similar to the modern adipocere values. They were also in line with the ^{13}C isotope composition of non-proteinogeneous human (animal) tissue or even steroids from humans (animals) living on a mainly C3 plant-based diet.

X-ray diffraction analyses showed that the child's body also contained inorganic substances, i.e. 95 percent by weight calcite ($CaCO_3$), 1 percent by weight dolomite ($CaMg(CO_3)_2$) and 5 percent by weight quartz (SiO_2).

The sarcophagus consisted of fine-grained, chalky, very light (5PB7/1) tertiary arkose with a large proportion of poorly rounded quartz grains cemented together with fine-grained material (Fig 5a–d). The part of the sarcophagus that was in direct contact with the corpse revealed a yellowish and brownish color (7.5 YR 7/4). Sarcophagus floor and lid samples contained silicone, oxygen and carbon, i.e. elements that are typically found in arkose sandstone (Fig. 5b); iron, magnesium, potassium and calcium were only found on the floor of the sarcophagus (Fig. 5c and d).

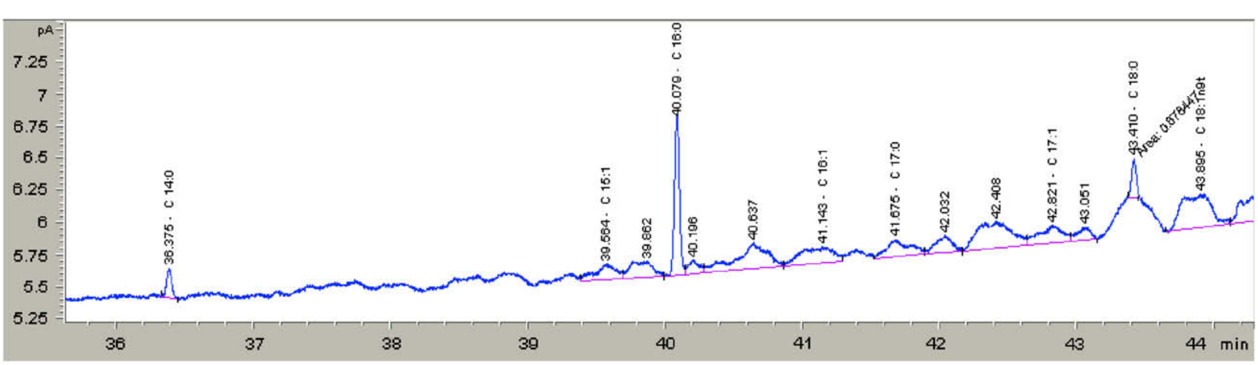

Fig. 3. Enhanced GC-FID chromatogram of the child's bone/tissue specimens (between 35 and 45 min).

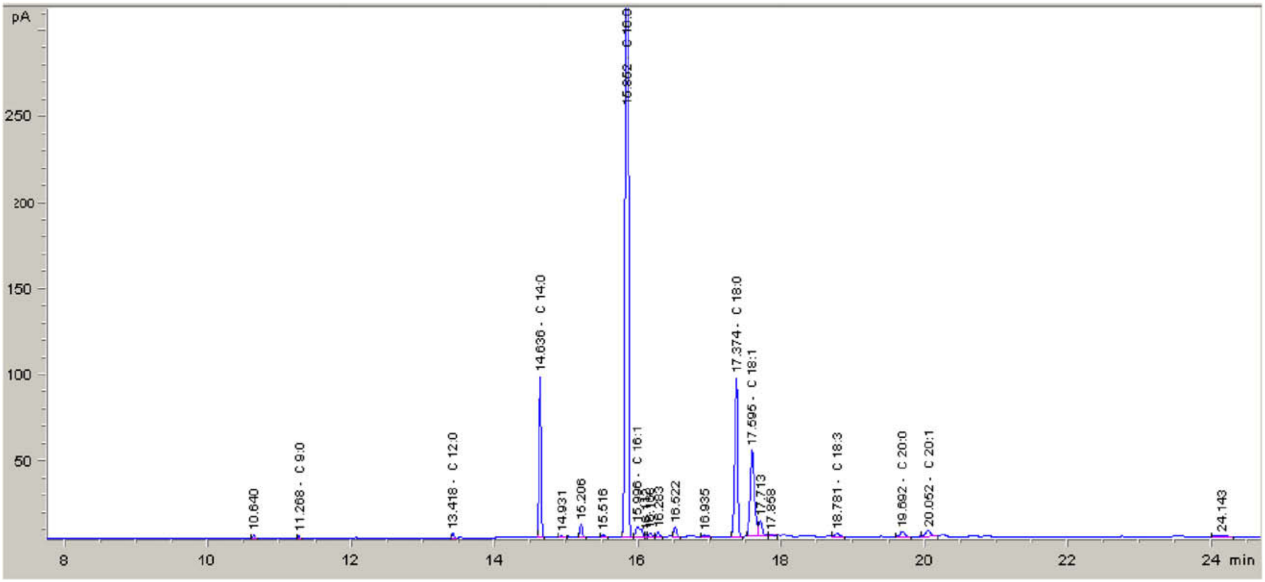

Fig. 4. GC-FID chromatogram of the FAME of modern adipocere on a polyethylene glycol column with a typical distribution of C-14:0, C-16:0 and C-18:0.

4. Discussion

Although the material only contained traces of fatty acids that are typical of adipocere, many other parameters substantiate the supposition that the crust is actually adipocere. The fatty acid composition of all specimens investigated differed considerably from that of human muscle tissues (Evershed and Connolly, 1988; Makristathis et al., 2002). Adipocere is characterized by elevated levels of C-16:0 fatty acid, which is potentially derived from C-18:1 fatty acid (Ruttan and Marshall, 1917; Morgan et al., 1983). The ratio of myristic (C-14), palmitic (C-16) and stearic (C-18) acids, the predominant acids found in adipocere (Forbes et al., 2005),

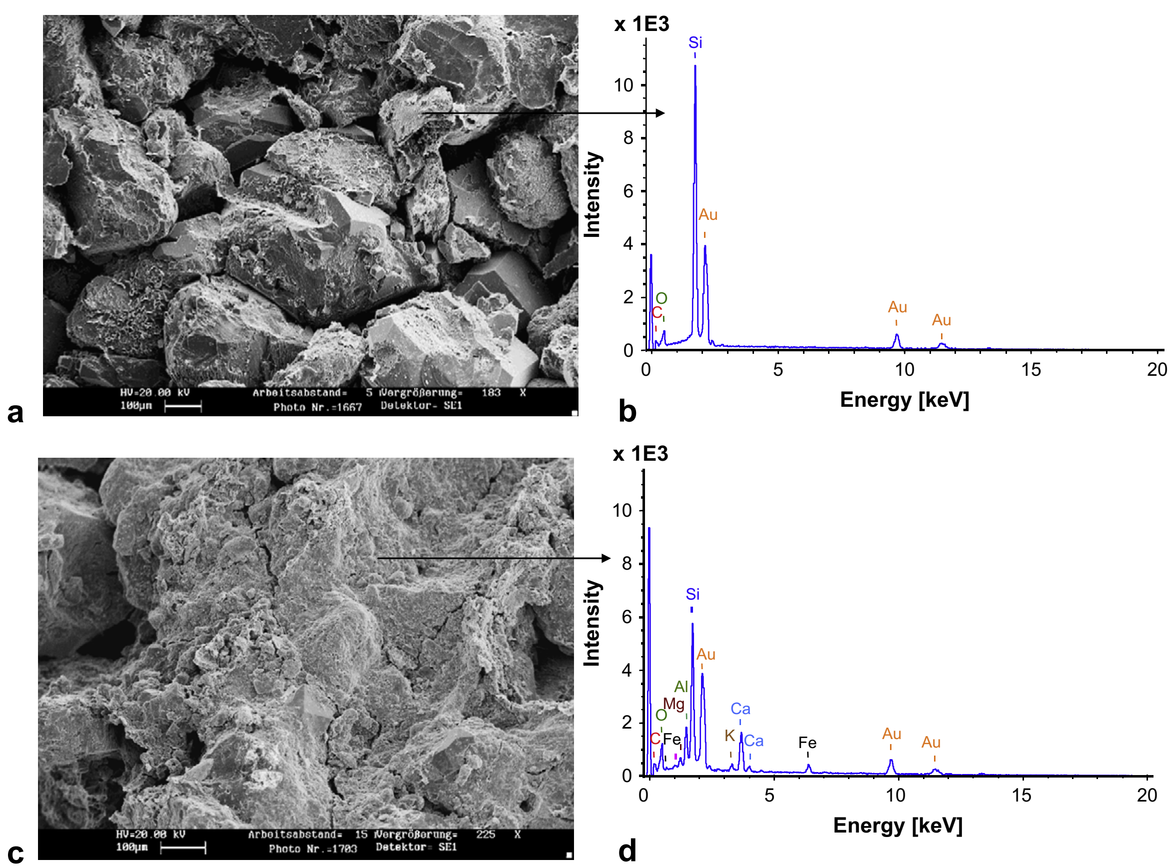

Fig. 5. a) SEM image of the sarcophagus lid; b) EDX image of the sarcophagus lid (outside), c) SEM image of the sarcophagus floor coating (inside), d) EDX image of the crust material (SEM image of the sarcophagus lid), e) SEM image of the crust material (Photos: M. Zarei).

supports the assumption that the child was covered in adipocere. The child's C-14, C-16 and C-18 fatty acid methyl ester ratios are similar to that of modern adipocere material and in good concordance with literature on fatty acid analyses of corpses in different soil samples (Forbes et al., 2002, 2005).

The body still contained some organic carbon. It must be assumed that these C components are very stable and resistant to microbes (=saturated hydrocarbons, Forbes et al., 2002), otherwise they could not have lasted for 1600 years.

Moist, anaerobic environments favor adipocere formation. In 1984, the corpses of around 200 people who lived in the area about 7000 years ago were discovered in an Indian burial ground (Windover Pond) (for more information see: http://www.nbbd.com/godo/BrevardMuseum/WindoverPeople/index.html, Stojanowski et al., 2002). The remains were buried in a peat area and were covered in adipocere. The corpses were extraordinarily well-preserved including even the brains (Doran et al., 1986). Bodies of those who have drowned are often also covered with adipocere (Cotton et al., 1987), as are corpses buried in moist and poorly permeable soils (Sticht et al., 1981; Mant, 1987). Corpses covered by clothing also favor the formation of adipocere (Mellen et al., 1993). In the case of the dead child, adipocere formation was most likely promoted by the following factors: (1) the sarcophagus was buried at groundwater level (2.9 m below the surface) and was hence frequently exposed to high water levels, and (2) pieces of clothing or bandages were also discovered on the body (Zipp, 2003). All these conditions promote the temporary lack of oxygen in the grave and hence adipocere formation. Moreover, the region has very chalky groundwater originating from the hinterland (carbonate rock from the young tertiary), which contributed to the high Ca^{2+} load of the environment (surrounding soil and groundwater) and excellent preservation of the bones, the calcite–dolomite crust and their hardening. Well-preserved bones are typical of a neutral or slightly alkaline environment (Keeley et al., 1977). Free Ca^{2+} ions in the soil solution or water passing through replace the protons of the H^+ ion-depleted hydroxylapatite of the bones, which leads to crystal stabilization and simultaneous weathering resistance (White and Hannus, 1983).

Saponification is mainly observed in adipocere on corpses found in water or soil with high Ca^{2+} concentrations (Döring, 1973). Sodium stearate exchanges its sodium ions for environmental calcium ions (Gill-King, 1997; Forbes et al., 2002), thereby forming Ca stearate, an insoluble product that also was responsible for the extreme robustness of the child's bones. The bandage fragments observed are assumed to come from the Roman custom of bandaging children like Egyptian mummies, and additionally the lime water ($Ca(OH)_2$) used to dissolve the flesh from the bones and get rid of the odor, a widespread funeral custom in Roman times (Cüppers, 1973), reacted with atmospheric CO_2 and/or CO_2 produced through microbial decomposition processes. The resulting calcium carbonate ($CaCO_3$) also contributes to the stabilization of the body's shape. It is not known whether such funeral customs favor the formation of adipocere. While it is not known how long adipocere takes to degrade (Fiedler and Graw, 2003), it is known that its degradation is impossible without oxygen (Froentjes, 1965). In the case under investigation, the fluctuating groundwater levels led to temporary anaerobic conditions, i.e. lack of oxygen. The sarcophagus was manufactured from arkose, a porous sandstone material that is typical of the 'Mainz Basin'. A band of continuous iron precipitations, i.e. Fe-(hydr)oxides, was found on the interior of the sarcophagus floor (Fig. 1) and can only be explained by changing redox conditions. Fe-(hydr)oxides are usually found at the boundary of anoxic and oxic environments that are typically found in areas with fluctuating groundwater levels. In particular, micro-crystalline Fe-oxides, such as those detected in the observed band, are generated through frequent changes of oxidation–reduction processes, in which reducing conditions prevent the crystallization of the Fe-(hydr)oxides. The fluctuation might explain the low content of organic carbon observed and the inability of bacteria to exert their full power to completely degrade the corpse and adipocere.

In accordance with previous papers, our case study highlights the extreme robustness of adipose tissues. The child is another one of many excellently preserved bodies where the cold or exposure to moistness promotes the preservation of bodies. The body found in the Similaun glacier in South Tyrol (Ötzi), is estimated around 5200 years old (Murphy et al., 2003; Bereuter et al., 1997; Bereuter, 2002). According to Bereuter, Ötzi's body must have been become covered in water around 100 AD when the climate in the region became warmer and the glaciers melted, thereby promoting adipocere formation and preventing the body from further decay. The animal and human bodies found in Thule, estimated to be around 3616 years old (Morgan et al., 1983), are another example where extreme cold and simultaneous or subsequent immersion in water, and hence oxygen deprivation, contributed to both preservation and adipocere formation.

Corpses/adipocere from biologically/microbially more active environments, for example soils and lakes, are usually a lot more recent than the previous examples. This clearly shows that biologically active environments favor the degradation of adipocere (e.g., temporal exposure to oxygen or the bodies being eaten by other organisms). Evans (1962) and Froentjes (1965) described cases in which adipocere was found in the soil after 127 or 140 years. In 1982, an adipocere-laden body was discovered in the Achensee mountain lake, Austria at a depth of 50 m (Makristathis et al., 2002). It is assumed that the body had been buried for about 50 years.

The well-preserved Late-Roman child is extraordinary, particularly considering that it was found in a relatively active environment. To our knowledge, this case is the only known example where fluctuating groundwater levels in soil have a) firstly contributed to the formation of adipocere and b) subsequently prevented the complete degradation of adipocere over a time span of 1600 years. Adipocere formation on corpses interred in sarcophaguses in late antiquity had not previously been mentioned in the literature. Reasons for this might be that (1) in contrast to mummification and brushit formation, this specific type of post-mortem degradation disorder was not recognized as such or (2) that the preservation conditions of our find were somewhat particular and favored the formation of adipocere. Therefore, the current study might contribute to the recognition of adipocere as another important type of post-mortem degradation disorder in future investigations.

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