

# Iceman's Mummification—Implications from Infrared Spectroscopic and Histological Studies

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**Abstract:** A skin sample from the Iceman (Ötzi, Similaun Man, Man from Hauslabjoch) was studied by means of IR spectroscopy and histology, and the results were compared to those obtained from nine other more recent human tissue samples with known case reports. Attenuated total reflection IR spectroscopy was used for studying the fate of proteins and lipids in these rare skin tissues. This technique provided a simple yet powerful means for semiquantitative determination of the main compound classes found in skin samples, namely, triacylglycerols, their

main breakdown products (fatty acids), and proteins. When combined with histology, IR spectroscopy provided reliable information about the main conditions under which mummification of the samples had taken place. In the case of the 5300-year-old Iceman, preserved collagen contributed to the conservation of morphological structures of the skin, although no

cellular structures such as nuclei survived. In addition, post-mortal alterations such as hydrolysis of triacylglycerols and phospholipids into fatty acids were unequivocally demonstrated. The solidified fatty acids provided a certain degree of preservation to the tissue characteristic of adipocere. Based on this observation, taken together with the concomitant loss of epidermis, we suggest that Iceman's body was submerged in water for a period of at least several months prior to desiccation. Results from other research disciplines support our conclusions.

## Keywords

adipocere · IR spectroscopy · lipids · mummification · skin

## Introduction

The mummified corpse of the Iceman (Ötzi) was discovered in September 1991 at 3210 m above sea level in a 20 m × 6 m chamber-like depression, below a rocky ledge, sheltered from the shearing flow of the glacial ice.<sup>[1]</sup> The trapped corpse was thus not expelled with the regular glacial turnover. A number of factors contributed to the corpse's preservation: the location, glaciological conditions, and estimated time of death (between end of summer and early autumn), as well as the circumstances leading to its discovery (the corpse was estimated to have emerged from the ice three days before discovery).<sup>[1]</sup> Calibrated radiocarbon dating of the body and artifacts was used to estimate the date of death at between 3350 and 3100 B. C.<sup>[2]</sup> This particularly well-preserved, late Neolithic, 45-year-old male,<sup>[3]</sup> who was found together with his belongings, provided a unique opportunity for interdisciplinary research.<sup>[4–6]</sup>

Iceman's skin was used for lipid analysis to investigate the process and circumstances of his preservation. Transformation of body fat into its partial decomposition products can be ana-

lyzed chromatographically and spectroscopically (TLC, HPLC, GC, GCMS, and NMR and IR spectroscopy).<sup>[7, 8]</sup> The two main types of natural mummification are desiccation and transformation into adipocere. The latter is a result of microbial activity converting the body fat into a grayish white lipid mixture, which consists mainly of saturated free fatty acids with an even number of carbon atoms, for example, myristic acid, the predominant palmitic acid, stearic acid, and 10-hydroxystearic acid.<sup>[9]</sup> Calcium salts of certain fatty acids may also be found in adipocere.<sup>[10]</sup> Triacylglycerols and unsaturated fatty acids like octadecenoic acid are only minor components of adipocere, but are more dominant in most desiccated tissues. Mummification (especially in glacial ice) may result from a combination of transformation processes, with superficial desiccated skin often containing adipocere underneath (detectable using gas chromatography).<sup>[8]</sup>

Prior to destructive analysis by GC, we characterized a sample of Iceman's skin using light microscopy, histology, and attenuated total reflection (ATR) IR spectroscopy. Histological trichrome staining was used to discriminate between the various tissue components in the partly decomposed material, and immunohistochemical staining for cytokeratins was used to demonstrate the loss of epidermis. IR spectroscopy provided information regarding the main compound classes typical for post-mortal skin by detecting their characteristic functional groups, such as esters within triacylglycerols, carboxyls within fatty acids, carboxylates within salts of fatty acids, and amides

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within proteins and ceramides. The reliability of our results was confirmed by means of ATR-IR spectra of several more recent tissues with known case reports, some of which were also analyzed by GC.<sup>[8]</sup>

In this study we present our results on the lipid and protein composition of Iceman's skin and nine other skin samples. Based on our findings, obtained by means of nondestructive ATR-IR spectroscopy and histology, we address some of the questions surrounding the mummification of the Iceman and discuss his likely submersion in water in the light of information obtained from other research disciplines.

## Experimental Section

**Samples:** The origin, age, and visual description of the samples is summarized in Table 1. Samples were divided into two groups according to their major lipid constituents. A-type samples contained mainly triacylglycerols, whereas fatty acids were predominant in B-type samples. All samples were stored at  $-20^{\circ}\text{C}$  prior to investigation.

A1: The thumb of one of the authors pressed onto the ATR crystal. A2: A post-mortem skin sample from the hip region recovered three hours following the death of a 50-year-old male as a result of cardiac arrest. A3: A freeze-dried portion of sample A2. A4: Skin from the upper arm of the corpse of a 53-year-old man discovered in his apartment 6 months following his estimated death in early spring. Primary mummification by desiccation preserved the epidermis (A4<sup>a</sup>) with the exception of a few liftoffs at small single spots (A4<sup>b</sup>).

B1: The scalp of A2 stored in water (with defined mineral content) at  $2^{\circ}\text{C}$  for 18 months. The sample was completely transformed into adipocere in which the hair became firmly rooted. B2: Tissue of the upper arm of a 30-year-old woman found after 50 years in a car at the bottom of lake

**Abstract in German:** *Eine Hautprobe des Eismannes (Ötzi, Similaun-Mann, Der Mann vom Hauslabjoch) wurde IR-spektroskopisch und histologisch untersucht und neun Gewebeproben mit bekannten Fallgeschichten gegenübergestellt. Die ATR (abgeschwächte Totalreflexion)-IR-Spektroskopie war die Technik der Wahl, um semiquantitative Informationen über den Lipid- (Triacylglycerine und Fettsäuren) und den Proteinanteil zu erlangen. Aus den spektroskopischen Daten lassen sich in Kombination mit den histologischen Untersuchungen zuverlässige Aussagen über die dominanten Bedingungen, unter denen die Mumifizierung der Gewebe erfolgte, ableiten. Im Falle des 5300 Jahre alten Eismannes hat die Erhaltung des Collagens maßgeblich zu einer Konservierung der morphologischen Strukturen beigetragen. Zellkerne und andere zelluläre Strukturen waren hingegen der Zerstörung preisgegeben. Weitere postmortale Zeretzungsprozesse wie der Verlust der Epidermis und die Hydrolyse der Triacylglycerine und der Phospholipide sind klar nachzuweisen. Die hydrolytisch freigesetzten Fettsäuren weisen für Fettwachs (Adipocere) charakteristische Veränderungen auf und lieferten als relativ inerte und stabile Festsubstanzen einen Beitrag zur Konservierung des Gewebes. Vor allem die Fettwachsbiildung, aber auch der Verlust der Epidermis zeigen, daß der Leichnam des Eismannes vor der Austrocknung zumindest einige Monate im Wasser gelegen ist. Die Resultate anderer Forschungsdisziplinen stützen unsere Schlußfolgerung.*

Table 1. Description of samples.

Sample	Site of find	Recovery after death (in years)	Description and references
A 1	–	–	author's thumb pressed onto the ATR crystal
A 2	–	–	skin from the hip, 3 h post-mortem
A 3	–	–	A 2 freeze-dried
A 3 <sup>a</sup> /A 3 <sup>b</sup>			outer surface/inner surface
A 4	apartment, bed	0.5	desiccated skin from the upper arm
A 4 <sup>a</sup> /A 4 <sup>b</sup>			epidermis present/absent
B 1	exp. water immersion	1.5	white adipocere from the scalp, stored at $2^{\circ}\text{C}$
B 1 <sup>a</sup> /B 1 <sup>b</sup>			outer scalp (containing hair)/inner scalp
B 2	Achensee, lake	50	white adipocere from the upper arm, $4^{\circ}\text{C}$ [8,11]
B 3	Stubai Alps, glacier	64	white adipocere from the lower arm with mineral deposits
B 4	Stubai Alps, glacier	57	white adipocere from the lower arm [8,11]
B 5	Ötztal Alps, glacier	29	white adipocere from the thigh [8,11]
C	Ötztal Alps, glacier	5300	skin from the hip, Figure 1 [4–6]
C <sup>a</sup> /C <sup>b</sup>			outer surface/inner surface

Achensee (Tyrol, Austria), 50 m below the surface where the water temperature is uniformly  $4^{\circ}\text{C}$ . Together with the torso, this tissue was transformed into adipocere, whereas the head, lower arms, and legs were completely skeletalized. B3: Lower-arm skin from the corpse of a 35-year-old man found in a glacier (Stubai Alps, Tyrol) 64 years following his disappearance. The head, one arm, and one hand were preserved by transformation into adipocere with minor areas of the skin also mummified by desiccation. A thin film of grayish mineral deposit coated most of his remains. B4: Tissue from the lower arm of the body of a 62-year-old man discovered 57 years after vanishing in the same region as B3. His head, torso, arms, one hand, and one leg were recovered from the glacier. These tissues were transformed into adipocere, and included some desiccated skin (e.g. at the chin with firmly rooted hair). B5: Tissue from the thigh of the completely recovered corpse of a 28-year-old woman found in a glacier (Ötztal Alps, Tyrol) 29 years following her death. The corpse was largely transformed into adipocere, and in some areas consisted of desiccated skin (with preserved hair at the scalp and pubis). C: A small skin sample (Figure 1) from Iceman's left hip, made available as a result of damage incurred by a jackhammer during recovery,<sup>[1]</sup> and stored under argon. With the epidermis lost the outer side of the skin (C<sup>a</sup>) appeared leathery. The inner side (C<sup>b</sup>) contained traces of subcutaneous fat.

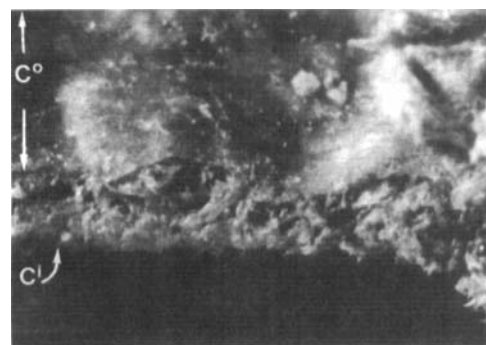


Figure 1. Iceman's skin ( $\times 15$ ) shown from above with the outer side of the reticular dermis (C<sup>a</sup>) and traces of subcutaneous fat below at the inner side (C<sup>b</sup>).

**Histology:** Routine formalin fixation of post-mortal disintegrated skin may result in the loss of the epidermis and was avoided. Freeze-dried samples were desiccated and repeatedly extracted for lipids with dry ethanol and xylol. Mechanical disturbance to surfaces during embedment and sectioning was minimized by using a double embedding method, combining celloidin and paraffin preparations.<sup>[14]</sup> Sections were stained by Goldner-Masson's trichrome method.<sup>[15]</sup> Immunohistochemical staining with anti-cytokeratin AE1/AE3 was used for detecting epithelial and mesenchymal cells (Boehringer Mannheim Biochemica).<sup>[16]</sup> Selective staining of squamous epithelium was accomplished with anti-cytokeratin #1 (Enzo Biochem).<sup>[17]</sup> Histological preparations were inspected by transmission and polarization light microscopy.

**Spectroscopy:** IR spectra were obtained on Perkin-Elmer Fourier transform IR spectrometers (models 1600 and 1740) equipped with triglycine sulfate detectors. Standard IR preparation techniques were used to obtain transmission spectra of reference compounds (liquid films between NaCl plates, solid films deposited on silicon, and KBr pellets). Nondestructive ATR technique (also referred to as internal reflection spectroscopy or multiple internal reflection) was routinely used for analyzing tissues, with the sample brought into close contact with the surface of the ATR crystal by applying moderate pressure. Spectra were obtained for the outer layers of the samples (the penetration depth, being wavelength dependent, is in the order of 1 to 10  $\mu\text{m}$ ) with a Perkin-Elmer Horizontal ATR Accessory equipped with a zinc selenide crystal. Sixteen scans with a spectral resolution of  $4\text{ cm}^{-1}$  in the spectral range of  $4000$  to  $650\text{ cm}^{-1}$  were averaged. Very flat and small samples yielded spectra that were partially overlapped by the spectrum of the vinylidene fluoride-hexafluoropropene copolymer of the pressure pad, where the significant spectral range was restricted to  $4000$  to  $1300\text{ cm}^{-1}$ . The spectra presented here have been normalized to a transmission value of 10% for the most prominent IR band.

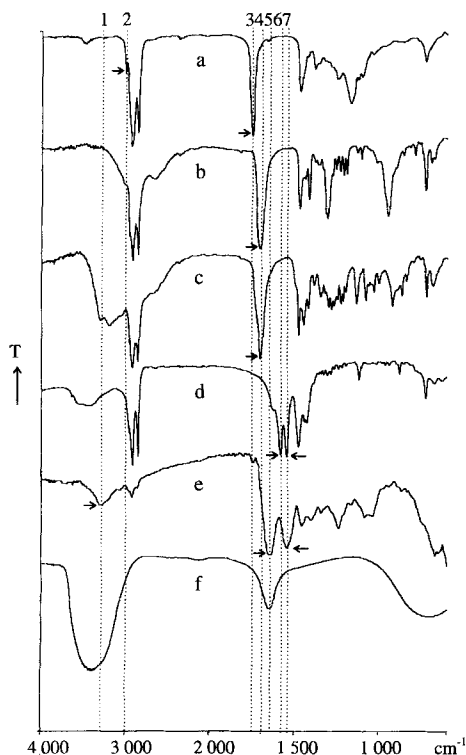


Figure 2. IR spectra of reference compounds: a) trioleate, b) palmitic acid, c) hydroxystearic acid, d) calcium palmitate, e) collagen, f) water. Dotted lines in combination with arrows indicate characteristic bands: 1) protein amide NH stretch (centered at  $\approx 3300\text{ cm}^{-1}$ ), 2) olefinic CH stretch ( $\approx 3010\text{ cm}^{-1}$ ), 3) triacylglycerol carbonyl CO stretch ( $\approx 1740\text{ cm}^{-1}$ ), 4) carboxylic acid carbonyl CO stretch ( $\approx 1700\text{ cm}^{-1}$ ), 5) protein amide I ( $\approx 1650\text{ cm}^{-1}$ ), 6) carboxylate CO stretch ( $\approx 1575\text{ cm}^{-1}$ ), 7) carboxylate CO stretch ( $1540\text{ cm}^{-1}$ ), d), as well as protein amide II ( $1540\text{ cm}^{-1}$ , e). The broad carboxyl OH band ( $3200$  to  $2400\text{ cm}^{-1}$ ) of fatty acids and the absorptions of water are not designated.

Evaluation of the IR spectra is based on characteristic absorption bands of suitable reference compounds: triacylglycerols (tripalmitate, tristearate, and trioleate), fatty acids (palmitic, stearic, hydroxystearic, oleic, and linoleic acids), calcium salts of fatty acids (palmitate and stearate), and proteins (keratins and collagens). Spectra of representative reference compounds are presented in Figure 2.

The characteristic spectral features that can reliably be used for an identification of the main compound classes are indicated. Distinction of water and amide NH stretching bands relies on absorptions at lower wavenumbers. The higher frequency carboxylate band shows an unusual splitting for crystalline calcium salts of fatty acids. In contrast to previously published work<sup>[18-21]</sup> we find that vibrational spectra are not capable of reliably distinguishing between the two major skleroproteins of dermis (98% collagen) and of epidermis (58% keratin).

## Results

**Recent human tissues:** The spectra of ante-mortem (A1) and post-mortem (A2) skin samples were similar (Figure 3). Both A1 and A2 yielded almost pure protein and water IR spectra,

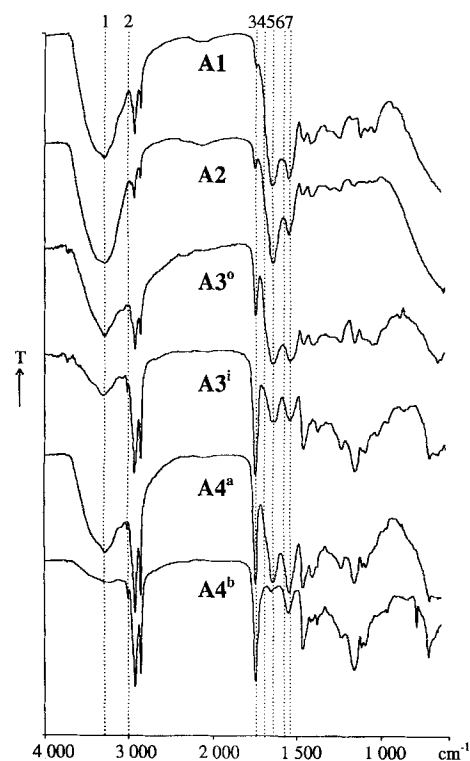


Figure 3. IR spectra of A-type skin samples.

with only very weak absorptions due to triacylglycerols. Olefinic CH absorption by unsaturated lipids was detected following the pressing out of liquids from A2. No gross structural degradation occurred in A3 (following the freeze-drying of A2); due to absence of water absorptions, both the amide NH stretching and olefinic CH bands became clearly discernible. The spectrum of the outer skin surface (A3<sup>o</sup>) was dominated by proteins, whereas that of the inner side (A3<sup>i</sup>) contained more triacylglycerols. A4<sup>a</sup> possessed all the skin layers (Figure 4), with the cornified layer (stratum corneum, arranged in a basketweave pattern rather than in the normal sheet arrangement) being well

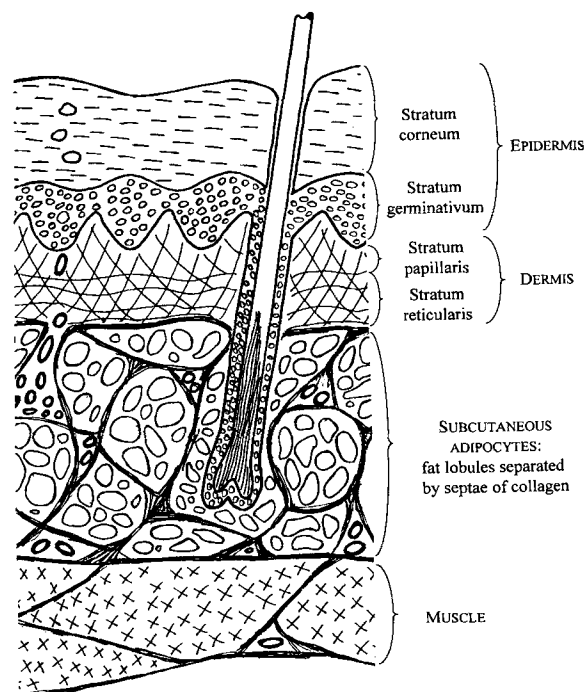


Figure 4. Structure of human skin.

differentiated from the basal layers (stratum germinativum) of the epidermis. Despite the partially degraded epidermis the basal layer cells were shown to be intact by monitoring

their nuclear integrity with chromatin staining (Figure 5). Chromatin-stained nuclei were also noted for the dermis, subcutaneous adipocytes, muscles, and glands. Epidermis preservation was immunohistochemically demonstrated by using anti-cytokeratin staining: AE1/AE3 for the basal layer of the epidermis (as well as glandular remains), and #1 specifically for the squamous epithelium with lost epidermis in A4<sup>b</sup> serving as control. Proteins and unsaturated and saturated triacylglycerols

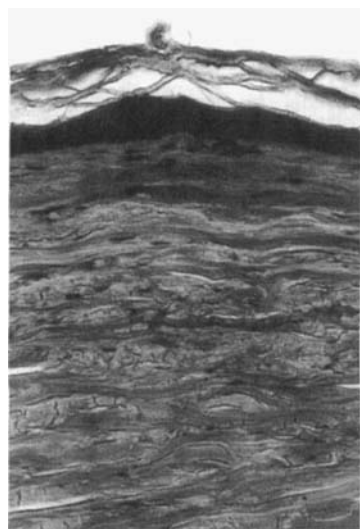


Figure 5. Trichrome staining of A4 showing the epidermis with part of the dermis ( $\times 320$ ).

predominated the IR spectra of A4<sup>a</sup> (Figure 3) with water and proteins appearing more abundant in this sample than in A4<sup>b</sup>. As a consequence of epidermis disintegration in A4<sup>b</sup>, the characteristic protein bands were almost totally absent. A4<sup>b</sup> closely complies with the pure trioleate reference spectrum, with the unique band at  $1550\text{ cm}^{-1}$  probably due to noncrystalline carboxylates.

IR spectra of B-type samples (Figure 6) differ from those of the A-type in that the lipids have been at least partly trans-

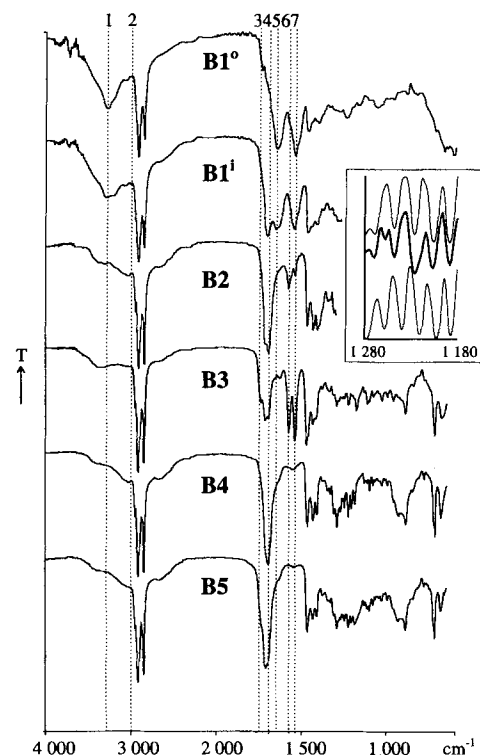


Figure 6. IR spectra of B-type skin samples. The insert displays the  $1280$  to  $1180\text{ cm}^{-1}$  range of B4, palmitic acid, and stearic acid (from top to bottom). The uppermost two patterns are nearly identical, but differ significantly from the lowest one.

formed into fatty acids, and their carbonyl CO bands have been shifted to lower wavenumbers as a result. Trichrome staining revealed that the stratified epithelium of the epidermis of B1 had disintegrated into a basketweave pattern, with no detectable chromatin. The epidermis itself had become completely detached from the dermis, remaining anchored to the latter by firmly rooted scalp hair. Remains of the epidermis were stained an intense red, whereas the papillary and reticular dermis layers below were clearly discernible by differential green staining of the collagen bundles. Hair follicles were demonstrated by staining the shafts for collagen (though chromatin staining was ineffective), with the fat tissue below the dermis showing its typical honeycombed pattern of fat lobules. Trichrome and anti-cytokeratin AE1/AE3 staining differentiated sweat glands from general adnexal structures and, combined with anti-cytokeratin #1, clearly displayed remnant epidermis. Despite its complete transformation into typical white and homogenous adipocere, B1 yielded different spectra for the outer (B1<sup>o</sup>) and inner sides (B1<sup>i</sup>) of the tissue. The spectrum obtained for B1<sup>o</sup> resembled that of pure protein, with only very weak indications for the presence of triacylglycerols and unsaturated components. The carboxyl OH band of fatty acids was clearly discernible, though that for the carbonyl CO was hidden by the strong amide I band. Reduced triacylglycerol and protein content in combination with abundant fatty acids (clearly evident from their characteristic carboxyl OH and carbonyl CO absorptions) in B1<sup>i</sup> resulted in their B-type classification. A minor signal on the high wavenumber side of the carbonyl CO band pointed to the presence of very small amounts of triacylglycerols, while another minor band demonstrated the presence of unsaturated components.

B2 to B5 showed several histological similarities, such as loss of epidermis and degradation of chromatin, muscles, vessels, nerves, glands, and hair follicles, with B2 additionally demonstrating loss of the dermis. Trichrome staining of B3 displayed woven collagen bundles within the reticular dermis as the outer layer. Samples B4 and B5 had loose collagen fibers resembling papillary layers above the reticular dermis. Subcutaneous fat lobules displayed signs of preservation in samples B2 to B5 and were separated by fibrous interlobular septae of collagen. Fungal spore-like granules were noted, with further anisotropic mineral particles detected on the reticular dermis of B3. Spectroscopically, the collagen differences seen with trichrome staining were not detected since proportionally very little protein was present. These spectra showed fatty acids without olefinic groups as the predominant components of adipocere, but no proteins. Small amounts of triacylglycerols were detected in B3 by a weak absorption band at  $1740\text{ cm}^{-1}$ , and with less certainty, in B5 by a corresponding weak shoulder (similar to B1<sup>1</sup>).

Being highly amenable to spectroscopic analysis, fatty acids can be individually identified. Thus, in B4 we accurately determined that palmitic acid rather than stearic acid was the primary component of the sample's adipocere (insert of Figure 6). Functional groups within fatty acids such as hydroxyl OH ( $3550$  to  $3400\text{ cm}^{-1}$ ) may also be detected, facilitating our putative identification of hydroxy fatty acids within adipocere (known constituents thereof<sup>[9]</sup>) and most prominent in B3. The band representing hydroxyl OH in B1 may have been hidden by the strong amide NH stretching band, since it was unambiguously detected chromatographically. Calcium salts of fatty acids (putative constituents of adipocere<sup>[10]</sup>) were detected in B2 and in larger amounts in B3.

**Iceman's skin:** Trichrome staining revealed collagen bundles arranged in parallel layers within the reticular dermis (Figure 7). As observed in the more recent samples, lobules of subcutaneous fat separated by fibrous interlobular septae of collagen were preserved, but nuclei and other cellular structures had been lost. Dense tubular structures, probably ducts of glands, were

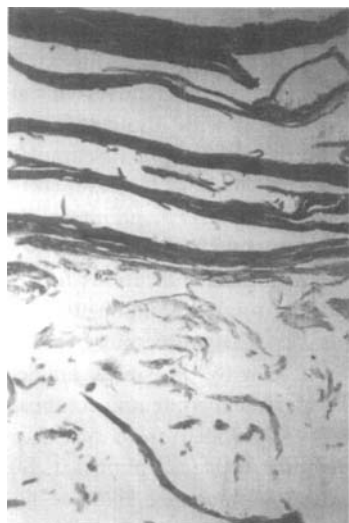


Figure 7. Trichrome staining of Iceman's skin showing the dermis with subcutaneous fat ( $\times 63$ ).

located in the subcutaneous fat. Polarization microscopy displayed anisotropic mineral particles on both surfaces of the sample. With the epidermis lost, no cytokeratin properties (or any signs of stratified squamous epithelium) were detected above the remaining reticular dermis.

IR spectra of the outer (C<sup>o</sup>) and inner (C<sup>i</sup>) side of Iceman's skin are shown in Figure 8. Bands due to proteins (amide I and II between  $1650$  and  $1500\text{ cm}^{-1}$  and amide NH stretch

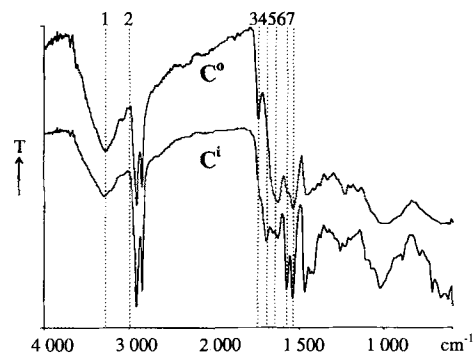


Figure 8. IR spectra of Iceman's skin.

at  $\approx 3300\text{ cm}^{-1}$ ) were clearly detectable in C<sup>o</sup>. Also observed were the following: a carbonyl CO band ( $1740\text{ cm}^{-1}$ ) for triacylglycerols, a weak band ( $3010\text{ cm}^{-1}$ ) for unsaturated groups, and some evidence of fatty acids ( $3000$  to  $2500\text{ cm}^{-1}$ ). As with B1<sup>o</sup>, the carbonyl CO band of fatty acids in C<sup>o</sup> was hidden. Similarly to the B1<sup>o</sup> and B1<sup>i</sup> samples, protein absorptions seen in C<sup>o</sup> were significantly reduced in the fatty-acid rich C<sup>i</sup> (note corresponding carboxyl OH and carbonyl CO bands). The two dominant carboxylate bands ( $1575$  and  $1540\text{ cm}^{-1}$ ) for calcium salts of fatty acids observed in B2 and B3 were also detected in C<sup>i</sup>. There was no indication for unsaturated components in C<sup>i</sup>, and triacylglycerols could only be estimated from a very weak shoulder at the high wavenumber side of the acid carbonyl band. From the above it would appear that some of the triacylglycerols within the layer of the surviving dermis remained unchanged, whereas those below were almost completely transformed into saturated fatty acids.

## Discussion

The histology of Iceman's skin confirmed the complete loss of the epidermis,<sup>[22, 23]</sup> in contrast to previous investigations claiming its preservation.<sup>[18–21]</sup> Furthermore, destruction of cellular structures may have taken place as a result of combined osmotic and other physical effects due to extensive water immersion, together with enzymatic, microbial, and chemical degradative processes. Nevertheless, dermal collagen fiber survival resulted in the preservation of the gross structure of Iceman's skin. Collagen is relatively resistant to both chemical and proteolytic degradation, and its breakdown by metalloproteinases requires normal body temperature for maximum activity.<sup>[24]</sup>

Iceman's corpse showed no major signs of putrefaction (e.g. preserved eyeballs and dermis), though his epidermis, hair, and nails were absent.<sup>[25]</sup> Normally associated with the early stages of this process,<sup>[26, 27]</sup> the observed loss of keratinous tissue may have been due to skin wrinkling (washerwoman's hands<sup>[28]</sup>), that is, disintegration of the epidermal basal membrane during water immersion resulted in detachment of the epidermis and its subsequent disappearance. We therefore propose that Iceman's corpse was first submerged in water for at least one<sup>[28]</sup> to three months and only then desiccated, since the reverse would have resulted in the diminished secondary swelling capacity of the connective tissue, with the subsequent preservation of the hair and nails.

Unlike other cellular components, lipids do not disperse in water but remain associated with tissues, though perhaps structurally altered. Fatty acids (the intermediate breakdown products of triacylglycerols and phospholipids) were more prominent in Iceman's skin than any other lipid component, proving a certain degree of lipid transformation and subsequent preservation. Spectra of the inner side of Iceman's skin ( $C^i$ ) resembled adipocere (B1 to B5) with fatty acids as the predominant lipids. Hydroxy fatty acids characteristic of adipocere<sup>[9]</sup> were detected by GCMS, but not by IR spectroscopy, since the high wavenumber range was covered by strong amide NH stretching bands. The lipid composition of  $C^i$  strongly indicated mummification due to adipocere formation, despite the sample appearing desiccated at first glance.<sup>[11]</sup> Closer inspection of Iceman's face (Figure 9) revealed accumulation of adipocere beneath the desiccated dermis, causing light-colored spots (état mammeloné<sup>[27]</sup>).

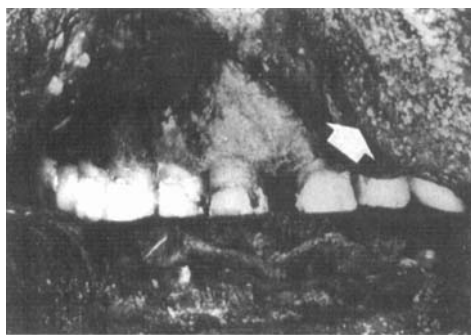


Figure 9. Iceman's oral area with typical light spots of "état mammeloné" at the left cheek (indicated by an arrow).

IR spectra of  $C^o$  and  $C^i$  indicated abundant proteinaceous structures in the outer layers, whereas lipoidal constituents predominated in the inner layer (cf.  $A3^o/A3^i$  and  $B1^o/B1^i$ ). This corresponds to the normal anatomy of this section of human skin (Figure 4), demonstrating a high degree of tissue preservation. Calcium salts of fatty acids were additionally present in  $C^i$  (cf. B2 and B3). Triacylglycerols remained relatively unchanged in  $C^o$ , while their hydrolysis into fatty acids in  $C^i$  was nearly complete. Furthermore, unlike in  $C^o$  unsaturated compounds were absent in  $C^i$ . GC analysis of four fractions of the Iceman sample demonstrated a declining gradient of triacylglycerols and unsaturated fatty acids from the outer to the inner side. This is in agreement with adipocere formation, which begins in the subcutaneous fat deposits and proceeds upwards to the neighboring tissues.

Transformation of human fat by microbes into adipocere usually takes several months and requires humid or waterlogged conditions as well as temperatures above freezing.<sup>[29,30]</sup> Based on our observations of loss of epidermis and formation of adipocere we conclude that the Iceman was immersed in water for several months. With the necessary precaution we list further evidence supporting our hypothesis:

- 1) A warm period during Roman times may have caused the ice containing the corpse to melt.<sup>[31]</sup>
- 2) Most of Iceman's belongings as well as his hair and nails were partly recovered below and slightly downhill of the corpse, indicating a water drift.<sup>[32]</sup>

- 3) His near-pure copper ax was postulated to have been in use and was covered by a water patina typically formed after extended exposure to a humid environment.<sup>[11]</sup>
- 4) The remarkably good state of preservation of Iceman's footwear, at the lowest point of the body (and furthest away from the water–air interface), indicates that the ice covering his feet had melted for a shorter duration than it had for the remainder of the corpse. This lends support to our hypothesis that portions of the corpse may have been exposed to water to a varying extent.

The list can be extended to include microbiological evidence:

- 5) The discovery of diatoms and cysts of a *Chrysophyceae* alga on Iceman's equipment further indicated water immersion.<sup>[33]</sup>
- 6) A *Micrococcus* species probably associated with human skin rather than clothing was found on Iceman's cloak and footwear. Epidermal loss and its subsequent translocation to the clothes may explain this finding.<sup>[34]</sup>
- 7) Two putative mesophilic fungi (T2709 and T44NS-4) were also found on Iceman's clothing suggesting a warm interval during his interment.<sup>[35]</sup>

Physical deformations incurred by the corpse further add to the list of evidence:

- 8) The Iceman was found lying face down with his head resting on an ice-covered rock. Particular deformations of soft tissue in his face and other secondary morphological changes, as well as the extensive shift of the left arm towards his upper right side describe a rotation of about 90° around the central axis of the body.<sup>[11]</sup> Though this was earlier proposed to be due to slight ice pressure,<sup>[3]</sup> we argue that the rotation from lying on his left to lying face down may have been effected by floatation in water (the usual posture of a freely floating body is face down). The facial deformations to his left side may have been due to his corpse being subsequently dragged over the rock as the water receded.
- 9) It is not likely that the skin lesion in the perital region of Iceman's head was due to predation since no feeding marks were observed.<sup>[3]</sup> Being the highest point of the corpse, this part of the head may have protruded from the water and had been exposed to the environment, where it sustained physical damage.
- 10) In forensic practice we observe that components of the cranial sutures disintegrate during prolonged water immersion. Iceman's skull deformations<sup>[36]</sup> may have been due to softened cranial sutures following water exposure.
- 11) Muscle-fiber contraction is inducible post-mortem; however, this was not demonstrated for Iceman, pointing to partial muscle decomposition. For this to occur, the corpse had to have been hydrated and soft.<sup>[37]</sup> In view of the climate at the site, thawing of the corpse would be most unlikely without the concomitant immersion in water.
- 12) DNA degradation noted for the Iceman<sup>[38]</sup> suggested that the corpse was not always frozen and protected from such DNA damage.

Taken together, the facts listed above point to the following probable scenario. Shortly after his death at the site of discovery in that prehistoric early autumn, Iceman's corpse was covered

by snow and ice. At some later stage, perhaps during Roman times, his sheltered corpse thawed and became immersed in the water collecting within the depression where it lay. During this brief warm period, most of the physical damages incurred by the corpse, as well as the formation of adipocere and loss of epidermis took place. In light of its excellent preservation, it would seem likely that desiccation of the corpse occurred shortly after its immersion in water. The desiccated remains were subsequently recovered from the ice in 1991.

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