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Lipid Preservation in Lindow Man

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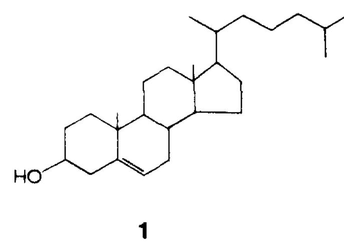

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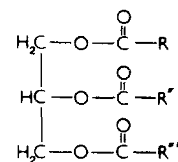

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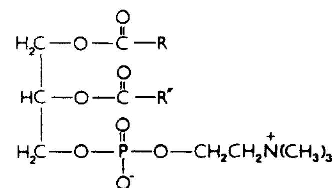

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The discovery of a well-preserved bog body, "Lindow Man" (ca. 2000 years old) [1], has provided a unique opportunity to study the degree and mechanism of preservation of a wide range of structural and physiological components. Although the cellular structure of bog body tissue is poorly preserved much of the collagen of the skin, teeth, bone, muscle tissue etc. has been preserved [2], presumably through a natural "tanning" process. For this to have occurred constituents of the surrounding peat water must have perfused the tissues. As part of our investigations into the preservation of the non-collagenous components of Lindow Man we have employed a combination of microanalytical techniques to determine the nature of lipoidal components. Detailed molecular analysis has allowed a clear distinction to be drawn between compounds endogenous to Lindow Man and those derived from the surrounding peat. Phospholipids and triacylglycerides appear highly degraded, although fatty carboxylic acids are readily detectable with a distribution similar to that of normal muscle. Significantly, endogenous cholesterol is well preserved despite microbial activity which has affected the phytosterols of the surrounding peat.

Lindow Man was not buried deep in wet peat like some Danish specimens [3] but deposited face-down in a pool of surface water [1, 2]. Hence, the normal processes of putrefaction were not immediately and completely inhibited by the acidic, sterile, anaerobic conditions of the peat-bog [1]. Destruction of the cellular structure of tissues has been by a combination of osmotic and other physical effects of waterlogging, together with microbial, enzymatic, and other chemical degradative processes. These degradative effects are reflected chemically, in part, by demineralization of bone, hair, and fingernails [2], and depurination, resulting in undegraded DNA being undetectable [4], although persisting in alkaline burials [5]. In contrast, other constituents such as high-molecular-weight glycosaminoglycans and glycopeptides from teeth [2, 6] and blood-group substances [2] are readily detectable. The aim of this investigation was to provide complementary information on the degree of lipid preservation in bog body tissue. As in previous organic chemical archaeological investigations GC [7] and GC/MS [8] were employed to derive detailed compositional information.

This study centers on a sample of psoas muscle part of which was used for investigations for intact DNA [4]. Maceration of the tissue with a chloroform/methanol mixture (2:1 v/v, 3 × 10 ml) [9] yielded a deep brown extract. The coloration is due to the presence of peat pigments and provides a clear indication that organic components of the peat have migrated throughout the body tissues. Analytical scale TLC (SiO_2; chloroform eluant) with visualization by sulphuric acid charring, revealed an intense spot corresponding to cholesterol (*1*, R_f 0.3). The substantial quantity (> 80 % of the neutral lipid) [10] of triacylglyceride (*2*, R_f 0.7) observable in healthy muscle, was undetectable. Cholesteryl esters (R_f 0.85), minor components of normal muscle tissue were also undetectable. A second TLC system [SiO_2, $CHCl_3/MeOH/H_2O$ (80:20:2) eluant] was used to screen for phospholipids (principal constituents, > 60 %, of the total fresh lipid). Although peat pigments showed as yellow/brown (R_f 0.95) and brown (R_f 0.49) spots subsequent visualization by exposure to iodine vapor, or sulphuric acid charring failed to reveal detectable amounts of phosphatidylcholine (*3*) and phosphatidylethanolamine, the most abundant phospholipid components of skeletal muscle [11]. These initial observations were consistent with intuitive expectations that lipoidal components bearing functional groups sensitive to the acidic environment would have been largely, if not completely, degraded during the 2000-year burial period. Both phospholipids (e.g., *3*) and triacylglycerols (*2*) contain ester moieties which would have been open to rapid hydrolysis, yielding long-chain fatty carboxylic acids, glycerol and, in the case of phospholipids, alcohol and phosphate groups. The latter three components, being water-soluble, would have been lost through dissolution and equilibration with the surrounding aqueous peat environment. In marked contrast cholesterol (*1*), a major component of fresh muscle (> 15 % of neutral lipid),

would be chemically stable and insoluble in the aqueous acidic interstitial waters of the peat bog. Capillary column GC analysis of the sterol fraction, isolated from Lindow Man lipids by preparative scale TLC, revealed only a single component corresponding in retention time to cholesterol. Subsequent GC/MS analysis confirmed this assignment. Parallel GC and GC/MS analyses of the sterols from the surrounding peat revealed characteristic Δ^5 phytosterols: sitosterol, stigmasterol, and campesterol. The presence of the corresponding stanols in higher abundance than the Δ^5 sterols, though with similar distribution, was indicative of the possibility that appreciable microbial activity [12] had once existed, and might still exist, in the peat bog. The presence of only a low abundance (ca. 1% of the total peat sterols) of cholesterol in the peat confirms that the sterol present in the bog body tissue was entirely endogenous. As only a trace (ca. 1%) level of cholestanol was detectable in the bog body tissue the endogenous cholesterol, unlike the peat sterols, had been largely unaffected by reductive processes during the period of burial.

Further GC and GC/MS analyses of the fatty carboxylic acids [presumed to arise largely from degraded triacylglycerides (*2*) and phospholipids (*3*)] of Lindow Man and the surrounding peat provided clear indications that although some migration had occurred this was at a low level. Fatty acids ($C_{12}-C_{30}$ chain-length range) occur in significant quantities in the Lindow peat (Fig. 1c). In contrast, the fatty acid distribution in fresh muscle is limited ($C_{12}-C_{20}$; Fig. 1a). Significantly, the distribution observed in Lindow Man (Fig. 1b) showed a strong resemblance to that of normal muscle (Fig. 1a). The presence of small amounts of higher acids, docosanoic and tetracosanoic, is indicative of low-level migration from the surrounding peat. The somewhat lower abundance of unsaturated components, compared with fresh muscle (cf. Fig. 1a, b), is not unexpected. Such compounds would have been relatively susceptible to oxidative degradation during the early period of submergence of the body, prior to the onset of anaerobiosis, and subsequently to microbial degradation. The enrichment of saturated fatty acids

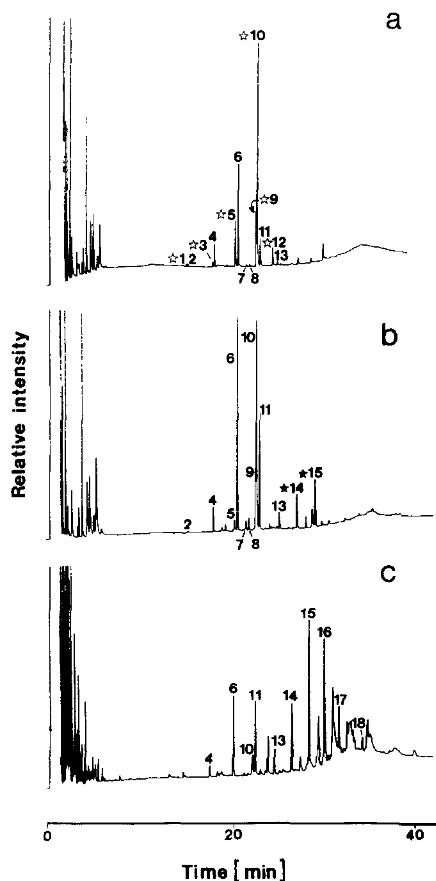

Fig. 1. Gas chromatograms showing fatty acids from a) fresh human muscle tissue, b) muscle tissue from Lindow Man, and c) Lindow peat as methyl ester derivatives. Fatty acids were obtained by treating aliquots of lipid extracts (5 mg) with methanolic sodium hydroxide (0.3 *M*, 1 ml, 30 min, 100 °C). Treatment with BF_3/methanol (14% w/v, 30 min, 60 °C) yielded fatty acid methyl esters. Peak identities are: *1* methyl dodecenoate (M^{+} 212, $C_{13}H_{24}O_2$); *2* methyl dodecanoate (M^{+} 214, $C_{13}H_{26}O_2$); *3* methyl tetradecenoate (M^{+} 240, $C_{15}H_{28}O_2$); *4* methyl tetradecanoate (M^{+} 242, $C_{15}H_{30}O_2$); *5* methyl hexadecenoate (M^{+} 268, $C_{17}H_{32}O_2$); *6* methyl hexadecanoate (M^{+} 270, $C_{17}H_{34}O_2$); *7* unknown; *8* methyl heptadecanoate (M^{+} 284, $C_{18}H_{36}O_2$); *9* methyl octadecadienoate (M^{+} 294, $C_{19}H_{34}O_2$); *10* methyl octadecenoate (M^{+} 296, $C_{19}H_{36}O_2$); *11* methyl octadecanoate (M^{+} 298, $C_{19}H_{38}O_2$); *12* methyl eicosatetraenoate (M^{+} 318, $C_{21}H_{34}O_2$); *13* methyl eicosanoate (M^{+} 326, $C_{21}H_{42}O_2$); *14* methyl docosanoate (M^{+} 354, $C_{23}H_{46}O_2$); *15* methyl tetracosanoate (M^{+} 382, $C_{25}H_{50}O_2$); *16* methyl hexacosanoate (M^{+} 410, $C_{27}H_{54}O_2$); *17* methyl octacosanoate (M^{+} 438, $C_{29}H_{58}O_2$); *18* methyl tricontanoate (M^{+} 466, $C_{31}H_{62}O_2$). *Open asterisks* (a) denote unsaturated fatty acids, constituents of fresh muscle lipid which have been substantially degraded in bog body tissue (b). *Closed asterisks* (b) correspond to fatty acids present in the bog body tissue which have migrated largely, if not entirely, from the surrounding peat. GC analyses performed on a Becker 407 using on-column injection (SGC, OCI II) onto a 12 m × 0.22 mm i.d. BP-1-coated (SGE, OV-1 equivalent, 0.25 μm film thickness) fused-silica capillary column using helium as the carrier at 100 cm s^{-1} and the oven temperature programmed from 50 to 300 °C at 8 °C min^{-1}. GC/MS analyses were carried out on a system comprising a Pye 204 GC linked to a VG 7070H double-focusing magnetic sector mass spectrometer. Data acquisition and processing were by a Finnigan INCOS 2300 data system

has been extensively investigated with respect to the formation of adipocere from animal and plant fats [13]. No adipocere was apparent in the remains of Lindow Man. However, the fatty acid composition of the muscle tissue suggests that processes of microbial alteration, analogous to those involved in adipocere formation, were in evidence in Lindow Man at some stage of submergence and burial.

In view of the high abundance (ca. 10% dry weight) of soluble organic matter present in the peat, it is remarkable that individual molecules and molecular distributions are so well preserved and readily recognizable. This level of preservation is clearly a reflection of the immobility of hydrophobic lipoidal components in the aqueous environment of quiescent peat. This is in marked contrast to the behavior of inorganic and ionic or polar organic constituents which rapidly dissolve and disperse by equilibration with the surrounding peat. In addition, the survival or degradation of specific molecules or molecular structural features serve as a sensitive indication of the physicochemical environment of the peat bog.

These observations have significant parallels with the principles of organic geochemistry, of which this investigation might be regarded as an especially unusual example. The study serves also

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to demonstrate further the role microanalytical chemical methodologies have to play in modern archaeological investigations.

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"Ordering" and Inclusion Phenomena of Bowl-shaped Particles in Deionized Suspensions

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Keen attention has been paid to the formation of "crystal-like" structures in deionized colloidal suspensions [1]. The ordering has been clarified both experimentally and theoretically. Recently, one of the authors described in detail the lattice structures of monodisperse spheres (specific gravity: 1.05 to 19.3) [2] and cylindrical particles [3] in deionized suspensions and in sedimentation equilibrium. From these experiments it was concluded that the ordered structure of the particles is caused by electrostatic interparticle repulsions and the elongated Debye-screening length around the particles which are counterbalanced by the force due to the gravitational field. Recently, mixed beds of cation- and anion-exchange resins, which can eliminate ionic impurities very effectively from the sample suspensions to levels as low as 2×10^{-7} *M* (due only to water dissociation), are available commercially. Thus, various distinct, extraordinary suspension properties are expected to occur, because the Debye-screening length for the completely deionized suspension is significantly long, in the order of micrometers. Because the asymmetric colloidal particles should be oriented to decrease the dead space as effectively as possible, the patterns of distribution are also expected to be asymmetric. Furthermore, the particles used in this work are bowl-like, and their inner cavities are expected to include guest spheres. These specific associations give rise to one of the excellent models for molecular recognition effects that are essentially important in biological systems such as substrate-enzyme binding and antibody-antigen binding. We now show these two features; ordered distribution and inclusion of a sphere into the cavity of a bowl-like colloid particle.

DR728 particle, a bowl-shaped colloid is a copolymer of styrene, methylmethacrylate, and methacrylate. The samples were purified by repeated decantations with deionized water. The size of the particles was determined using an electron microscope. The outer and inner diameters were 9 μm and 6 μm, respectively. The monodispersity in their sizes was rather high. Polystyrene spheres of DlA35 (diameter: 2.95 ± 0.13 μm, charge density: 14.2 μC cm^{-2}) and DlA12 (2.02 ± 0.014 μm, 6.2 μC cm^{-2}) were products of Dow Chemical Co., N1000 (1.02 ± 0.02 μm, 2.6 μC cm^{-2}) and N800 (0.78 ± 0.09 μm, 1.2 μC cm^{-2}) were purchased from Sekisui Chemical Co. (Osaka). The samples of DR728 and the spheres were treated on a mixed bed of cation- and anion-exchange resins (Bio-Rad, AG501-X8(D), 20 – 50 mesh) for at least ten days. The water used for the purification and for suspension preparation was deionized by using cation- and anion-exchange resins (Amberlite IR-120B and IRA400) and was further purified by a Milli-Q reagent grade water system (Millipore Co., Bedford, MA).

The particles were photographed with a reversed-type metallurgical microscope (Axiomat IAC, Carl-Zeiss, D-7082 Oberkochen, FRG). The suspension cell was made from a glass tube (height 7 cm, diameter 2 cm) which had a cover glass at one end. An ordered distribution of the particles appeared within several hours after 10 ml of the suspension was introduced into the cell, and reliable data for the system in sedimentation equilibrium could be obtained after 7 days. The height of the sedimentation layer was less than 1 mm.

Microscopic observation was made through the cover glass at the bottom of the cell. A typical picture of DR728