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Exceptional preservation of a prehistoric human brain from Heslington, Yorkshire, UK

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ABSTRACT

Archaeological work in advance of construction at a site on the edge of York, UK, yielded human remains of prehistoric to Romano-British date. Amongst these was a mandible and cranium, the intra-cranial space of which contained shrunken but macroscopically recognizable remains of a brain. Although the distinctive surface morphology of the organ is preserved, little recognizable brain histology survives. Though rare, the survival of brain tissue in otherwise skeletalised human remains from wet burial environments is not unique. A survey of the literature shows that similar brain masses have been previously reported in diverse circumstances. We argue for a greater awareness of these brain masses and for more attention to be paid to their detection and identification in order to improve the reporting rate and to allow a more comprehensive study of this rare archaeological survival.

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1. Introduction

In August 2008, a human skull containing the remains of a brain was discovered in a waterlogged pit at Site A1, Heslington East, York, UK (Fig. 1). The excavation, directed by Mark Johnson of the York Archaeological Trust (YAT), was undertaken for the University of York ahead of construction of their new campus (Johnson, 2008; Dean, 2008). A multi-disciplinary team was brought together to investigate the brain and the circumstances of its preservation. The survival of brain tissue in human remains may be expected where the biodeterioration of soft tissues has been inhibited, whether through deliberate mummification or particular conditions of the burial environment (Cockburn et al., 1998; Aufderheide, 2003). Familiar examples include the desiccated sand burials and embalmed mummies of Ancient Egypt (David, 1997; Karlik et al., 2007; Lewin and Harwood Nash, 1977); the deeply frozen bodies of the Franklin expedition (Beattie and Geiger, 1987; Notman et al., 1987), the 5000 year-old Tyrolean Ice Man (Hess et al., 1998; Spindler, 1993) and Inca mummies of the high Andes (Ceruti, 2004); the tanned bog bodies from across Northern and Western Europe (Brothwell and Gill-

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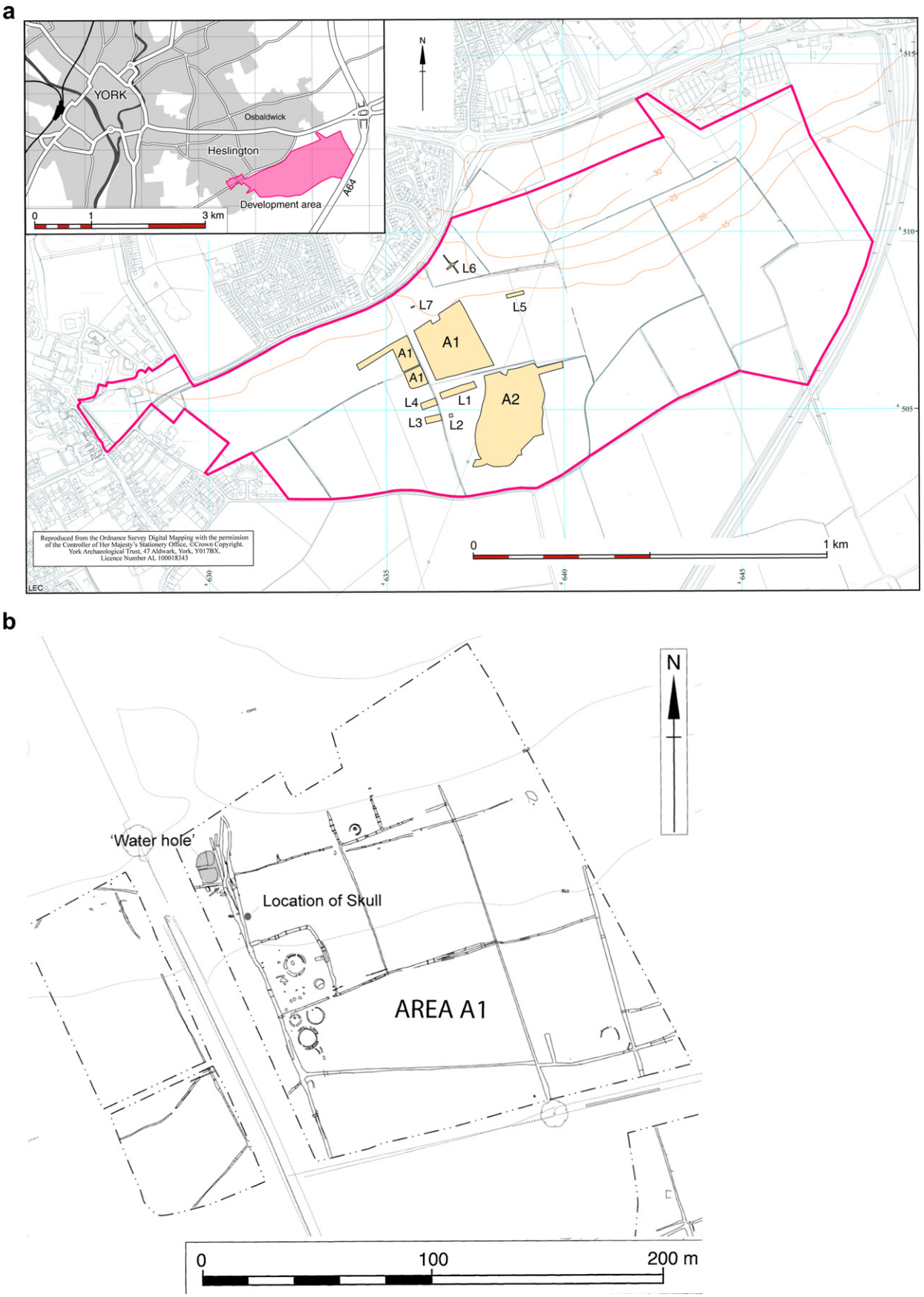

Fig. 1. Heslington East. a, Location of the campus development and excavations, and b, detail of Area A1 and the pit containing the skull (York Archaeological Trust).

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Robinson, 2002; Spatz et al., 1958); bodies sealed in lead-coffins, such as St Bees man (Tapp and O'Sullivan, 1982); and crypt burials such as those at Spitalfields Church, London (Adams and Reeve, 1987). In all these cases, where brain tissue persists, there is survival of other internal and external soft tissues, such as lung, heart, muscle, skin and hair, unless they were deliberately removed in antiquity. However, in the Heslington case, brain survives in the absence of other soft tissues.

Here we report the archaeological circumstances of this remarkable find, the recording and sampling of the brain masses, macroscopic, radiographic and histological observations on the masses, and the results of a literature search to locate other examples of brain survival in skeletalised human remains, from which we discuss the conditions that appear to favour preservation. Subsequent papers will report in detail the biomolecular analyses that are ongoing in order to characterise the surviving material in more detail. The overall aim of the project is to understand the taphonomic trajectory that led to exceptional preservation, and hence to determine whether particular aspects of the death and inhumation of this individual may have contributed to the survival of what is ordinarily a most vulnerable soft tissue.

This paper has been compiled by Sonia O'Connor (PI) based on reports from and discussions with the numerous colleagues engaged in the investigation of this specimen, and revised by her and Terry O'Connor. Specific contributions to the investigations reported here were as follows:

Identification and recovery of the brain, critical review of previous cases of brain survival in excavated skeletons and the characteristics of adipocere – Sonia O'Connor (University of Bradford).
3D Photography and laser scanning – Anthony Masinton (University of York).
High-resolution laser scanning – Philip Dodds and Andy Warriner (Konica Minolta).
CT and MRI scanning – Phil Duffey and David King (York Hospital).
Examination and recording of the skull – Jo Buckberry (University of Bradford), Terry O'Connor (University of York).
Sediment thin-section – Raimonda Usai (University of York).
Scanning Electron Microscopy – Andrew Wilson (University of Bradford).
Bone thin-section – Holger Schutkowski (University of Bradford).
Soft tissue thin-section – John Denton (University of Manchester).
DNA – Keri Brown (University of Manchester).
Brain neurochemistry – Axel Petzold (University College London).
Tissue and sediment chemistry – Salim Al-Sabah, Danish Anwar, Ed Bergström, Stephen Buckley, Matthew Collins, Adam Dowle, Karl Heaton, Brendan Keely, Matthew Pickering, Kirsty Penkman, Martin Rumsby, Kimberley Shackleton, Jerry Thomas, Jane Thomas-Oates (University of York), Esam Ali, Howell Edwards, Andy Gledhill, Carl Heron (University of Bradford), Konrad Dorling, Elsa Correia Faria, Peter Gardner (University of Manchester).

The project was funded by the University of York and English Heritage.

2. Material

The Heslington East campus of the University of York lies to the south-east of the city (grid ref SE636506), and is constructed on former agricultural land. The northern edge of the site is marked by an east–west trending glacial moraine, from which the land falls on a gentle southward slope. The subsoil parent material is a heterogeneous mix of mostly glaciofluvial silts, sands and gravels. Survey and excavation work was undertaken in 2003–2008 in advance of construction. A number of former water channels were identified, together with linear ditches of prehistoric (mostly Iron Age) date, consistent with the drainage of water from springs and seepage along the moraine slope. One such spring had been adapted into a series of well points, two of which had wicker linings, sited at the junction of a number of ditches and apparent land boundaries, and including posts and wattle indicating it to have been managed from the Bronze Age through to the middle Iron Age. To the south of these features were a dozen or so pits whose contents were not typical of occupation waste, hinting at some more ceremonial function that persisted from the Bronze Age through to the early Roman period. Many were marked by a single stake, and their non-trivial contents included 'burned' cobbles of local stone. In addition the headless body of a red deer *Cervus elaphus* had been deposited in a palaeochannel, one of the earliest features of the site, and an unworked red deer antler was found in an Iron Age ditch.

In one of the pits, a dark-stained human cranium with articulated mandible lay approximately face-down with no other contents, in a matrix described as a moist, friable to plastic dark brown organic rich, soft sandy clay with occasional beige sand flecks/spotting (context 2619) (Fig. 2). The skull and a small number of animal bone fragments were recovered from 2619. After recovery of the skull, it was noted to contain a resilient mass not consistent with inwashed silt. Inspection of the endocranial cavity through the foramen magnum showed the presence of 'a quantity of bright yellow material' (R. Cubitt, pers. comm.). Subsequent endoscopy (Fig. 3), radiography of the cranium and examination of a sample of the mass, identified it as consistent with brain masses previously seen in skeletalised human remains from the Hull Magistrates' Court site in Kingston-upon-Hull, UK (O'Connor, 2002). The cranium, with its articulated mandible and vertebrae C1 and C2, and a possibly unrelated single intermediate phalanx, was removed to cold storage pending further imaging and extraction of the brain masses. DNA sequencing of samples from the brain gave a nearest match in haplogroup J1d, a recently defined haplogroup, first seen in modern DNA sequences from just a few individuals from Tuscany and the Near East. So far it has not been identified in Britain but further sampling in British populations may yet find this haplogroup. Alternatively it may have existed in the past in Britain and been lost through genetic drift. A calibrated radiocarbon date of 673–482BC was obtained from collagen extracted from the mandible (OxA-20677: 2469 ± 34 bp).

Fig. 2. The skull as found (York Archaeological Trust).

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Fig. 3. View of the brain through the foramen magnum using endoscopy (Sonia O'Connor).

3. Methods

3.1. Recording of the skull and brain

Following removal of only the most superficial adhering sediment, the cranium was imaged by (i) digital photography (including 3D) and low-resolution laser scanning, to give a detailed record of its external condition prior to opening the cranium to access the brain mass, and (ii) by conventional medical CT and MRI, to record the position and internal structure of the brain mass (Fig. 4). Adhering sediment from the eye sockets and the exterior of the cranium was sampled for geochemical analyses. The skull was then recorded and measured according to standard osteoarchaeology protocols (Buikstra and Ubelaker, 1994; Brickley and McKinley, 2004). A conventional autopsy saw-cut was made around the cranium a little above the temporal line, allowing most of the parietal bones and parts of the frontal and occipital bones to be lifted away in one piece. The brain masses could be seen surrounded by sediment in the occipital portion of the endocranial space (Fig. 5). The brain masses were removed, and then cleaned using a small, blunt, plastic spatula to lift away lumps of sediment and a soft, fine, paintbrush and a gentle stream of distilled water to clean the surfaces of each mass. Sediment from the intra-cranial space was retained, and the cranium, mandible and cervical vertebrae were further washed and examined. A full photographic record was made throughout, including low-power photomicroscopy and video. A 3D photographic record was made of the brain masses immediately following cleaning, and the brain masses and elements of the skull were scanned with a high-resolution 3D laser digitising system, the Konica Minolta Range 7. The bones were allowed to air-dry slowly, and were then re-examined for signs of trauma or other evidence pertinent to death and deposition. Light microscopy and SEM, carried out under low-vacuum conditions in an environmental chamber without coating or otherwise preparing the material, were employed in the examination of detail.

3.2. Sampling

Sampling of the brain was restricted as much as possible to one of the masses for the sake of consistency and to preserve the anatomically distinctive portions for future study. The piece selected for sampling retained some external morphology and also areas of fractured surface, revealing the interior, but was large enough to provide sufficient material for all the proposed analytical procedures. Separate samples were taken for a range of biomolecular analyses and to be fixed for histological study. The sample sizes were appropriate to the minimum required for each analytical protocol (in the range of 20 mg–2 g). The inwashed sediments and the bone of the cranium were also sampled.

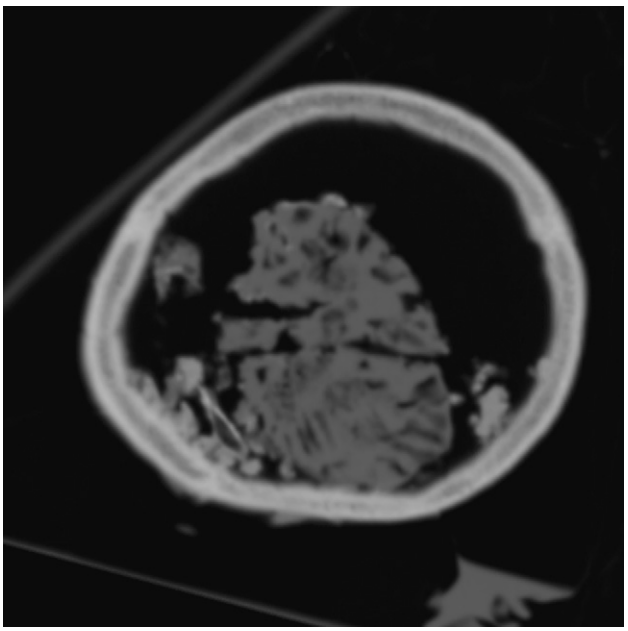

Fig. 4. CT section of the cranium showing two of the larger fragments, which may be the cerebral hemispheres separated by the sagittal cleft (David King).

Fig. 5. The brain remains and sediment in situ in the opened cranium. Two of the larger masses are indicated by the arrows (York Archaeological Trust).

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3.3. To characterise the internal and external deposits on the cranium

Sediments internal and external to the cranium were examined by light microscopy and SEM, carried out as above. Samples were also taken for biomolecular analyses. A coherent lump of sediment from the interior of the cranium was sampled for micromorphological analysis. The liquid phase was gradually replaced with acetone, then with a styrene-rich resin (Crystic 17449). After two months curing, 30 μm thin sections were cut from the block and examined using polarized light microscopy and the criteria defined by Stoops (2003).

3.4. To investigate the state of preservation of the bone

To gain an overview of the state of preservation of the bone, a loose fragment, approximately 10 × 15 mm, was taken from close to asterion on the left side of the cranium. This fragment was embedded in epoxy resin, cut by microtome to 70 μm thickness, polished and mounted. The sections were viewed by transmitted light at 200–400× magnification.

3.5. To explore the surviving morphology and histology of the brain

Low-power reflected light microscopy was undertaken to characterise surfaces of the surviving tissue. SEM was carried out as above. For histological analysis, tissue fixed in neutral buffered formalin (pH 7.1) was dehydrated through alcohol, cleared in xylene, then embedded in paraffin wax. The block was then microtomed to give 5 μm thin-sections, which were mounted onto glass microscope slides. The sections were stained with toluidine blue at pH 4.0, then with haematoxylin and eosin. TEM was undertaken by post-fixing the formalin-fixed tissue in osmium tetroxide, embedding it in araldite resin and staining the sections with lead citrate and uranium acetate.

3.6. To explore the chemistry of the brain

The range of biomolecular analyses in progress is directed towards testing the brain masses for characteristic brain chemistry, and investigating the composition of the apparently stable material to which the brain has undergone taphonomic alteration. Only preliminary results are presented here. Highly sensitive neuroimmunological techniques, together with proteomic analyses, have demonstrated the presence of a range of brain-specific proteins, mainly of structural nature (Petzold, 2005). We are currently investigating how these proteins survived and what insight they may give on the circumstances between death, the burial environment and preservation of the Heslington brain. An analysis of DNA from the brain and from the skull has also been undertaken. Full results of all of these analyses will be reported in subsequent papers.

4. Results

4.1. Examination of the skeletal remains

Anthropological examination showed the skull to be that of a male, following criteria outlined by Buikstra and Ubelaker (1994) and Bass (1995). Based on Meindl and Lovejoy's (1985) criteria for cranial suture closure, and on Brothwell's (1972) method for molar attrition, an age-at-death estimate of 26–45 years was obtained, most likely in the younger half of this range. Cranial measurements show the cranium to be unexceptional in size and shape, with no significant evidence of disease. Examination of the two associated vertebrae showed the arch of C2 to be fractured on either side of the centrum (Fig. 6). The fracture surfaces are consistent with peri-mortem fracture, and their location is consistent with a traumatic spondylolisthesis of C2. A cluster of c. 9 transversely-directed fine cut-marks made by a thin-bladed instrument, such as a knife, are visible on the anterior aspect of the centrum of C2. SEM examination confirmed these to be peri-mortem (Fig. 7). Fine cracks on the cranial base and disruption of the right temporal suture are mostly post-mortem, but three appear to be peri-mortem – two to the occipital and one to the greater wing of the sphenoid. The traumatic spondylolisthesis and the cut-marks are consistent with death by an abrupt trauma to the neck, followed by deliberate and careful dismemberment of the head between C2 and C3.

4.2. Preservation of the bone

Although superficially well preserved, the skull has obviously undergone loss of organic matter, making it necessary to resample to obtain sufficient collagen for radiocarbon dating. That said, microanatomical features are very well preserved: lamellar and osteon structures are readily recognized, and osteocyte lacunae can

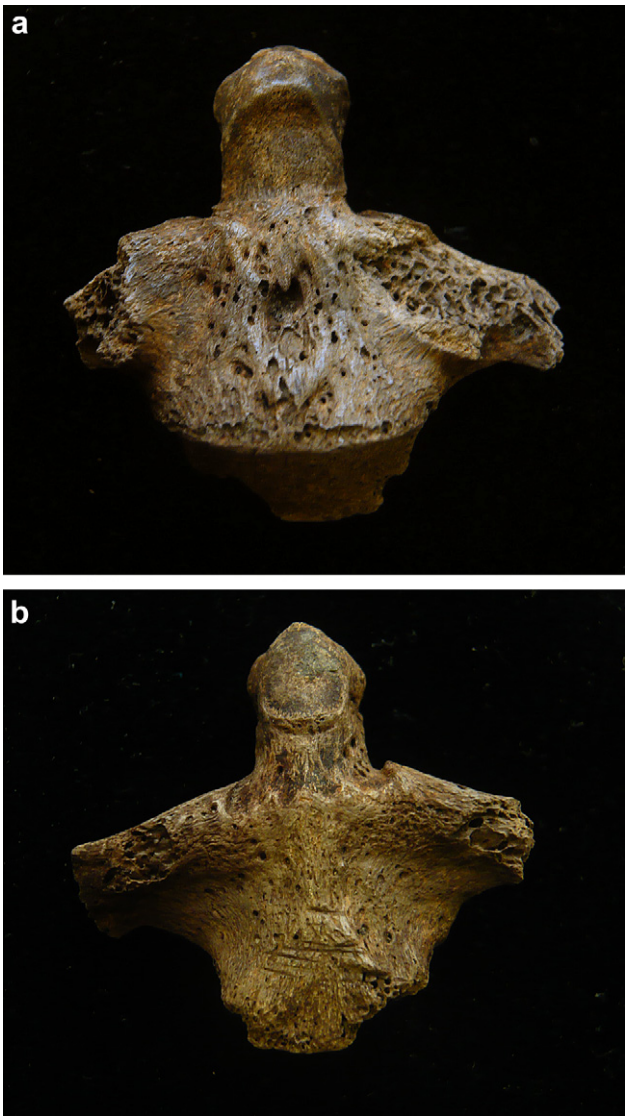

Fig. 6. Associated vertebrae C2. a, Peri-mortem damage and b, anterior aspect showing cut-marks (Jo Buckberry).

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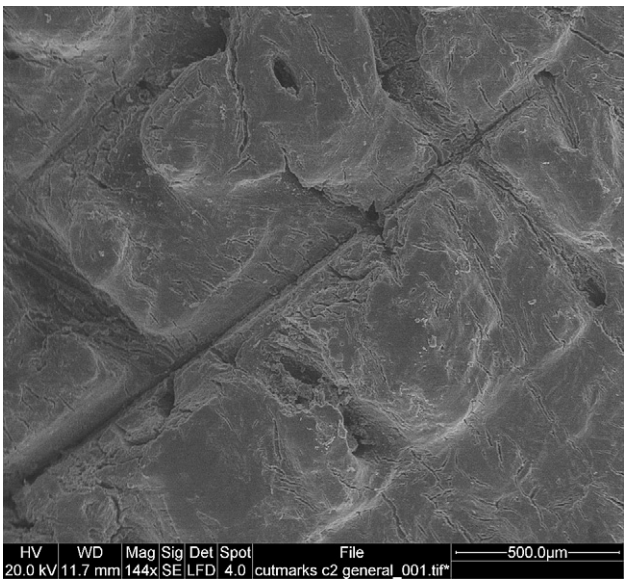

Fig. 7. SEM of the peri-mortem cut-marks on the anterior aspect of C2 (Andy Wilson).

be resolved throughout the sections (Fig. 8). Brown staining, possibly from humic compounds in the burial environment, is strongest at the surface of the bone, fading towards the interior. Most significantly, there is no trace of microbial activity, bacterial or fungal, with none of the porosity or 'tunnelling' that is characteristic of putrefactive microorganisms (Jans et al., 2004). However, in places the bone displays linear cracks and erosive patterns to the reversal lines of osteons, structural damage that has been interpreted as the result of intermittent periods of dry and moist-saturated burial environments (Piepenbrink and Schutkowski, 1987; Smith et al., 2002).

4.3. Deposits within the skull

Micromorphological examination showed an unsorted, predominantly quartzose, sediment, with low grain weathering, no textural pedofeatures, and only a few traces of randomly-arranged plant tissues. The results show a very low degree of pedogenesis acting on the sediment. The lack of pedofeatures indicates little movement of water within the sediment after its deposition within the skull, and little or no movement of decomposition products through voids or through the matrix.

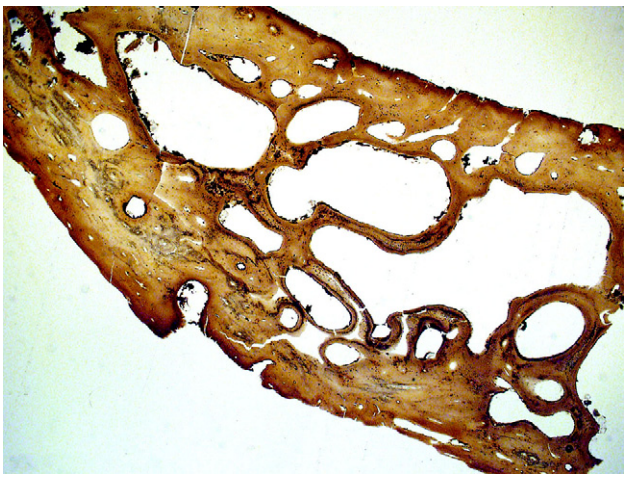

Fig. 8. Thin-section of the bone of the skull indicating the overall very good preservation but also structural cracks in the compact bones and around Haversian systems (Holger Schutkowski).

4.4. Examination of the brain masses

CT of the cranium had revealed several fragments of brain loose inside and mixed with the denser sediment. The brain masses had recognisable sulci and gyri and many internal voids which are mainly post-mortem features (Fig. 4). CT could not differentiate between the brain cortex (grey matter) and underlying medulla (white matter). Subsequent MRI produced similarly useful images but did not elucidate this point.

Within the cranium, there were five major brain masses and many millimetre-scale fragments of brain tissue. Following superficial cleaning of the masses, gross anatomical features such as well-defined sulci and gyri were clearly identifiable. The tissue was odourless, had a smooth surface with a resilient, tofu-like texture and was more pink/brown or tan in colour in daylight than had appeared when first viewed by electric light within the cranium, when it had appeared yellow (Fig. 9). The scale of the surface convolutions, taken with the overall volume of surviving material, indicated that the brain had shrunken to perhaps 20% of the volume of a fresh brain (i.e. to about 250–300 ml). One of the largest masses, approximately 70 mm × 60 mm × 30 mm, also had an area of black membranous material, perhaps a fragment of the meninges (Fig. 9b). Where the masses had fractured, they had a soft, granular texture and were lighter in colour than the exterior surfaces (Fig. 9c). The expected distribution of white and grey matter could not be discerned macroscopically.

Imaging by 3D laser scanning of the major fragments was successful, except for one fragment that, when turned over, distorted to the extent that the data sets could not be integrated. The images reconstructed from the data allow the fragments to be viewed from all angles and brought together in different combinations to help identify the portions of the brain that have survived, without the risks associated with handling the fragments themselves. The photographs can also be digitally added to the surface of the 3D reconstructions (Fig. 10) and the scan data could be used to produce replicas of the fragments using rapid phototyping techniques. Imaging by micro-CT was unsuccessful but it is hoped that more useful results may be obtained in the future if the results reported here, and subsequent analyses of composition, allow a more precise calibration of equipment to optimise the imaging.

4.5. Histological examination of the brain masses

Both toluidine blue and haematoxylin–eosin staining of the brain sections showed a homogeneous, amorphous substance that had not retained any cellular or matrix structure. TEM also did not detect any surviving cellular structure although these images did show the presence of numerous morphologically-degraded structures characteristic of the myelin sheath of nerve fibres (Fig. 11). A few bacterial spores could be recognised on TEM, but no other traces of putrefactive bacteria or fungi where evident. This observation is more consistent with degradation by sterile autolysis than with putrefaction. SEM captured the spongy, granular nature of the fracture surface of the brain mass (Fig. 12) but added little to the understanding of the surviving histology.

4.6. Biomolecular analysis of the brain masses

It is only possible here to provide a summary of the initial results of the array of qualitative, quantitative and compositional

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Fig. 9. Brain fragments, after cleaning. a, Surface convolutions and b, meninges on mass A, and c, fracture surface on mass C (Sonia O'Connor).

techniques that have been applied to the brain. The C:N ratio of the tissues was 6.3 ($n = 2$) suggesting considerable retained nitrogen, more than double the nitrogen content of the least refractory soils, (soil C:N ratios range from 13 to 40; e.g. Aitkenhead and McDowell, 2000). Degraded protein and indications of possible cyanobacterial colonisation were identified and the proportion of proteinaceous matter in the brain was higher than in the sediments in and around the skull. Notably, however only 5% of the total tissue in the Heslington brain was detectable as hydrolysable amino acids whereas proteins represent more than a third of the dry weight of fresh brain tissue. The remaining nitrogen revealed by the C:N ratio remains unaccounted for in terms of protein. The amino acid profiles were all remarkably similar to each other and racemization levels were all lower than D/L 0.06 except for Asx (D/L 0.17). However when compared with a fresh brain the material was depleted in polar amino acids (Asx, Glx, Ser) and enriched in hydrophobic amino acids (Gly, Ala, Val, Phe, Leu, Ile). Two brain-specific proteins were unambiguously identified using proteomic techniques; myelin proteolipid protein (lipophilin) and claudin-11 (oligodendrocyte-specific protein). The three lipophilin peptide sequences matched are common to humans and a number of other mammals; the single claudin-11 peptide sequence detected is present in both humans and orang-utan. Aggregated structural brain-specific proteins have been isolated using highly sensitive in-house developed immunoassays (for review see Petzold, 2005).

Lipids constitute almost half the dry weight of fresh vertebrate brain tissue and roughly 25% of the total free cholesterol in the whole body (McIlwain and Bachelard, 1985), however, very little undegraded solvent-soluble brain lipid appears to have been preserved and this brain contains lower proportions of extractable lipids (0.8–1.1% wet weight compared with 17.1% for rat brain) than the sediments from the interior of the skull, the maxillary sinus and orbits. Significantly there is an almost complete absence of phospholipids and only a trace of cholesterol, but coprostanone (5β-cholestan-3-one), a well-known microbial alteration product of cholesterol, was detected along with fatty acids and other degradation products of a wide range of lipids including hydroxyfatty acids, aldehydes, thiophenes and very low levels of sterols/stanones. This includes a series of 2-hydroxyfatty acids, identified as trimethylsilyl derivatives, with carbon numbers ranging from C22:0–C25:0 with the 2-hydroxyfatty acid of C24:0 predominating. The latter molecule is also known as cerebronic acid and is the major hydroxyfatty acid found in brain cerebrosides (Eng et al., 1965). The 2-hydroxy derivative of C24:1 is also present in the lipid extract albeit in lower abundance compared to fresh brain tissue. Cerebrosides are present mainly in brain white matter, especially in myelin (Siegel and Albers, 2006, 35). The same distribution of 2-hydroxy acids and sterols has been found in the brain tissue of Gristhorpe Man (Melton et al., 2010; Heron, unpublished results) and in permafrost-preserved mammoth brains (Kreps et al., 1981).

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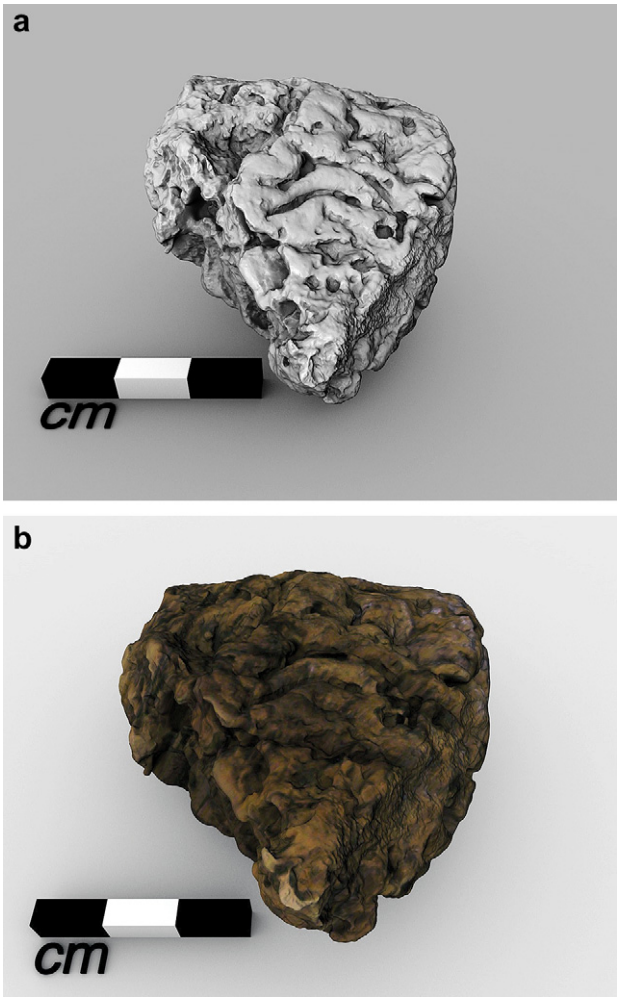

Fig. 10. Brain fragment (mass A) reconstructed from 3D data. a, rendering of Konica Minolta laser scan data and b, with photo textured rendering (Anthony Mastinson).

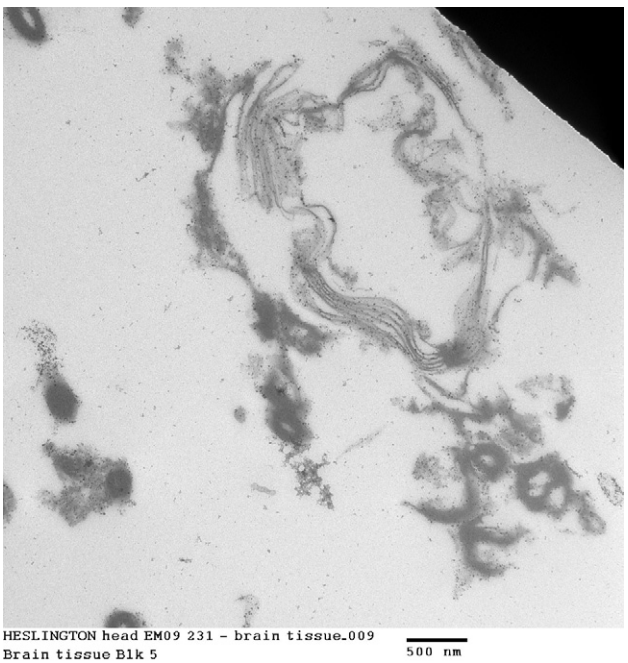

Fig. 11. TEM of myelin structure preserved in the brain tissue (John Denton).

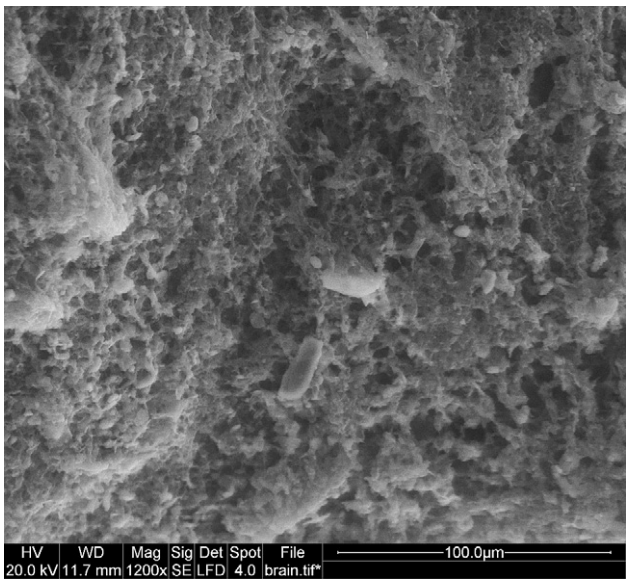

Fig. 12. SEM of a fracture surface of the brain tissue (Andy Wilson).

There are no biomarkers indicative of artificial preservation techniques such as smoking or embalming in the Heslington brain mass. Fatty acids are not a major component of the lipid extracts and there is no evidence for the formation of adipocere, since none of its characteristic products, such as steroid degradation products (Adachi et al., 1997), were detected. Instead, high-resolution mass spectrometry and sequential thermal desorption and pyrolysis GC–MS, together with TLC, indicate that a high molecular weight, long chain, hydrocarbon material now forms a major hydrophobic component of the brain mass. Research in progress aims to characterise this material.

5. Discussion

The aim of drawing together these results so far is to bring this remarkable specimen to the attention of the research community. Two questions are central to any discussion. First, was there anything particular about the circumstances of death and deposition of this head that may have predisposed the degradation of the brain to a more stable material that retains so much of the original morphology? Second, just how exceptional is this survival?

5.1. Death and deposition

It is rare to be able to postulate the cause of death for skeletonised human remains of archaeological origin. In this case, we have evidence for a traumatic spondylolisthesis of the neck and for subsequent, almost surgical, dismemberment of the head. If the former was not fatal, the latter certainly would have been. Knife cuts on the axis indicate decapitation by means of a thin-bladed knife, inserted from the anterior aspect (i.e. from the front of the throat) and repeatedly pulled transversely across the neck. It is unlikely that this could have been undertaken on a conscious individual. This sequence of events would have separated the head from the circulatory system and thus from the internal organs from which endogenous putrefaction would spread, and would also have allowed blood to drain from the detached head. Open wounds are associated with an increased rate of putrefaction but burial may have followed quite rapidly, reducing infection by exogenous putrefactive organisms and slowing decomposition considerably (Fiedler and

Graw, 2003). Below 21 °C the activity of most putrefactive bacteria would have been inhibited, and in ground temperatures a few degrees above freezing it could have been a week before the first signs of putrefaction occurred (Mant, 1987). Certainly, the bone histology is inconsistent with any putrefaction of skeletal tissues, and the same appears to be the case for the brain tissue itself.

The results are inconsistent with post-mortem curation. The characteristic signs of early putrefaction are absent from the bone histology and the chemistry of the surviving brain masses is inconsistent with any deliberate intervention such as smoking. Furthermore, it is unlikely that deliberate curation through smoking or desiccation would have preserved brain tissue in the absence of any other soft tissues. Indeed, desiccation is likely to have led to rapid collapse of the brain tissues. There are no superficial markings on the skull indicative of excarnation, so it is improbable that a more completely-preserved head was deliberately stripped of surviving skin and muscle before interring the skull. Despite the place that 'trophy heads' appear to have played in Iron Age societies and evidence for curation of human remains in the Bronze Age (Aldhouse-Green, 2002; Parker-Pearson et al., 2005), there is no evidence that this case is anything other than a decapitated head in which post-mortem putrefaction was rapidly inhibited. That inhibition, we argue, was likely achieved through rapid burial into a fine-grained wet sediment. This is a distinctive and unusual sequence of events, and could be taken as an explanation for the exceptional brain preservation. However, similar preservation has been noted in other cases where decapitation clearly did not occur (Beriault et al., 1981; Clausen et al., 1979; Dailey et al., 1972; Doran et al., 1986; Flinn, 1989; Haneveld, 1984; Oakley, 1960; O'Connor, 2002; Pääbo et al., 1988; Papageorgopoulou et al., 2010; Pilleri and Schwab, 1970; Royal and Clark, 1960 and Tkocz et al., 1979). What, if anything, does the Heslington case have in common with these others surviving brains?

5.2. Yet another unique ancient brain?

Even a brief survey of the forensic literature confirms that brain tissue is normally expected to decompose rapidly in the immediate post-mortem period and survival of brain when other soft tissues have decomposed is reported as exceptional, as in the case of the dried brain found in a skull by police in the bushveld near Radfontein, South Africa (Eklektos et al., 2006) or even 'unique', as in the case of several examples recovered from a mass grave in Bulgaria, buried 45–50 years previously in loose, stony soil (Radanov et al., 1992). However, here and in Appendix 1, we summarise the results of a literature search that shows the survival of brain in otherwise skeletalised remains from waterlogged sediments to be unusual but by no means unique.

Perhaps the earliest published accounts of this type of preservation come from pre-Revolution France. Between December 1785 and October 1787, the crowded and foetid cemetery of St Innocents' Church was cleared as a public health measure (Sourkes, 1992). Contemporary accounts report that brain matter was found in large numbers of otherwise skeletalised bodies even where the skull was ruptured by soil pressure (Thouret, 1790). A substance likened to 'white cheese', and known as gras or grave wax, that had formed over some of the bodies preserving the shape of the flesh, and organs such as the brain, heart and liver was subsequently named 'adipocere' (Fourcroy, 1791). Significantly, Thouret noted that, even at the earliest stages of decomposition, the brain always showed less deterioration than other soft tissues and was often the last to survive. The best-preserved examples came from those common graves in which adipocere frequently covered the bones. These brains had recognisable hemispheres and convolutions, filling about a quarter to a third of the cranial cavity, even after 20 or 30 years burial. These brain masses were pulpy, soft and 'fusible' between the fingers, although some were firmer, more solid and looked more friable. Outwardly they had the colour of fresh brain but internally the colour varied, some with a differentiation between the whitish medullary substance (white matter) and a covering of greyer cortex (grey matter). The brains were odourless, the only smell being that of the adipocere surrounding the bones. Brain masses were also found in individual interments in which adipocere had not formed. In drier contexts such as charnel houses, surviving brains were described as very small and blackened on the surface but whiter within and very hard. Thouret (1791) also cites earlier reports of brains preserved in burials, several recorded in the 17th century including a corpse of 50 years' burial with a brain that was white, oily and odourless; several from Avignon being moist, soft and apparently un-deteriorated; and the survival of soft and apparently un-deteriorated brains from a large number of executed men thrown into a well and only retrieved 80 years later. Fourcroy was so intrigued by the unexpected preservation of brain tissue that he embarked on a detailed study of human and other animal brains, laying the roots of the modern study of neurochemistry (Fourcroy, 1793).

Appendix 1 lists examples of brains in otherwise skeletonised remains reported in the literature since 1960. The specimens from Florida are well-known, and all derive from a range of water-saturated environments (Beriault et al., 1981; Clausen et al., 1979; Dailey et al., 1972; Doran et al., 1986; Pääbo et al., 1988; Royal and Clark, 1960). The Zihl Canal specimens are important as they were not deliberate inhumations, but the victims of a flood that in 1 BC collapsed a wooden bridge and immediately buried all (Pilleri and Schwab, 1970). Rapid burial was also inferred for the specimen from Scole, the context and body position of which led Turner-Walker (pers. comm.) to suggest furtive burial, as with the victim of a murder. Many of the cases listed in Appendix 1 are from cemeteries associated with medieval monastic houses (Flinn, 1989; Haneveld, 1984; O'Connor, 2002; Tkocz et al., 1979). This may be nothing more than a chance association: medieval cemeteries constitute a high proportion of the burials excavated in northern Europe. However, an association with wet or waterlogged sediments is very clear.

The treatment and investigation afforded these brains have varied considerably, making systematic comparison quite difficult. A number were immediately fixed in formalin, several have been subjected to conventional histological section and staining, and only a few have any chemical characterisation. CT examination of the well-preserved specimens from Kingston-upon-Hull (O'Connor, 2002) confirmed internal structures consistent with brain, albeit distorted and reduced in volume (Figs. 13 and 14). In most cases, the specimens were recognised as brain masses largely, it would seem, because they retained the characteristic surface convolutions, albeit in shrunken form, and were located in the intra-cranial cavity. In the cases of Droitwich (Oakley, 1960) and Quimper-Bretagne (Papageorgopoulou et al., 2010), the survival of the brain in the form of adipocere has been proposed. We think this unlikely, for reasons discussed below.

In addition to the specimens listed in Appendix 1, publicity surrounding the Heslington brain has brought to our attention other recent examples. Stephen Rowland, Oxford Archaeology North, has provided details of a 19th century example from a coffin burial in waterlogged clay at a cemetery in Blackpool, Lancashire, UK, and Jonny Geber, Margaret Gowen & Co Ltd., Heritage and Archaeology Consultants, informed us of several in various states of preservation (wet, moist and dry) from a Great Famine (1845–52) mass grave at the site of the former union workhouse in Kilkenny City, Ireland. A news report that has not yet led to academic publication describes three 1800 year-old brains from skeletons excavated from heavy, moist clay at the site of Aoya-Kamijichi, Tottori Prefecture, Japan (Science 27 April 2001: 617. http://www.sciencemag.org/cgi/content/short/292/

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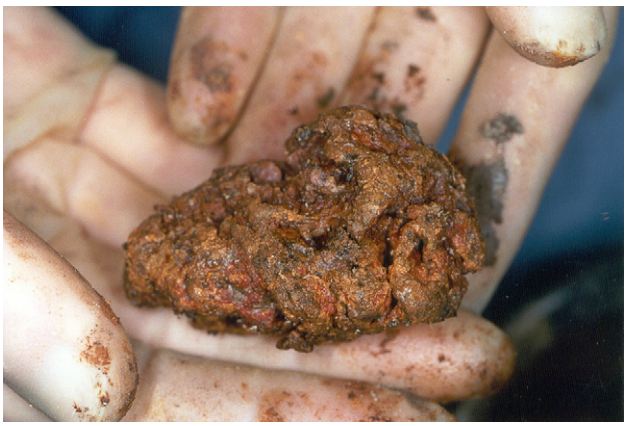

Fig. 13. Brain fragment (SK 1789) from the Humberside Archaeology Unit excavation at the Hull Magistrates' Court site, Kingston-upon-Hull (York Archaeological Trust).

5517/635d). Add to these accounts anecdotal evidence from archaeologists, and the survival of brain remains, although still uncommon, might well be expected in submerged sediments and waterlogged burials.

5.3. Common factors for preservation

The Heslington brain and all the published examples cited have been preserved in waterlogged, anoxic deposits as demonstrated by the survival of wood and other organic plant remains. Preservation is independent of the presence of a coffin or clothing and seems to occur under an apparently wide range of pH conditions, but where the sediment of the burial is recorded, a clay component is often specifically mentioned. There is only occasionally the slightest trace of any other soft tissue preservation on the skeletal remains. Apart from the ambiguous example from Sandwell (Flinn, 1989), all the brains were similar in colour and texture when wet, were odourless and appeared to be shrunken, typically to 25–50% of the original volume. They have not been preserved by mineralisation and there was no evidence of natural tanning or deliberate embalming. The skulls are mostly intact, but there are exceptions, and no evidence of insects, arthropods or other macro-organisms involved in the decomposition of buried corpses have been reported. Where the condition of the bone is mentioned it appears to be in a good state of preservation but is often stated to be darkly stained. As with the examples from Paris described by Thouret (1791), these archaeological examples display a range of preservation, from complete miniature brains to amorphous, fibrous masses, probably reflecting

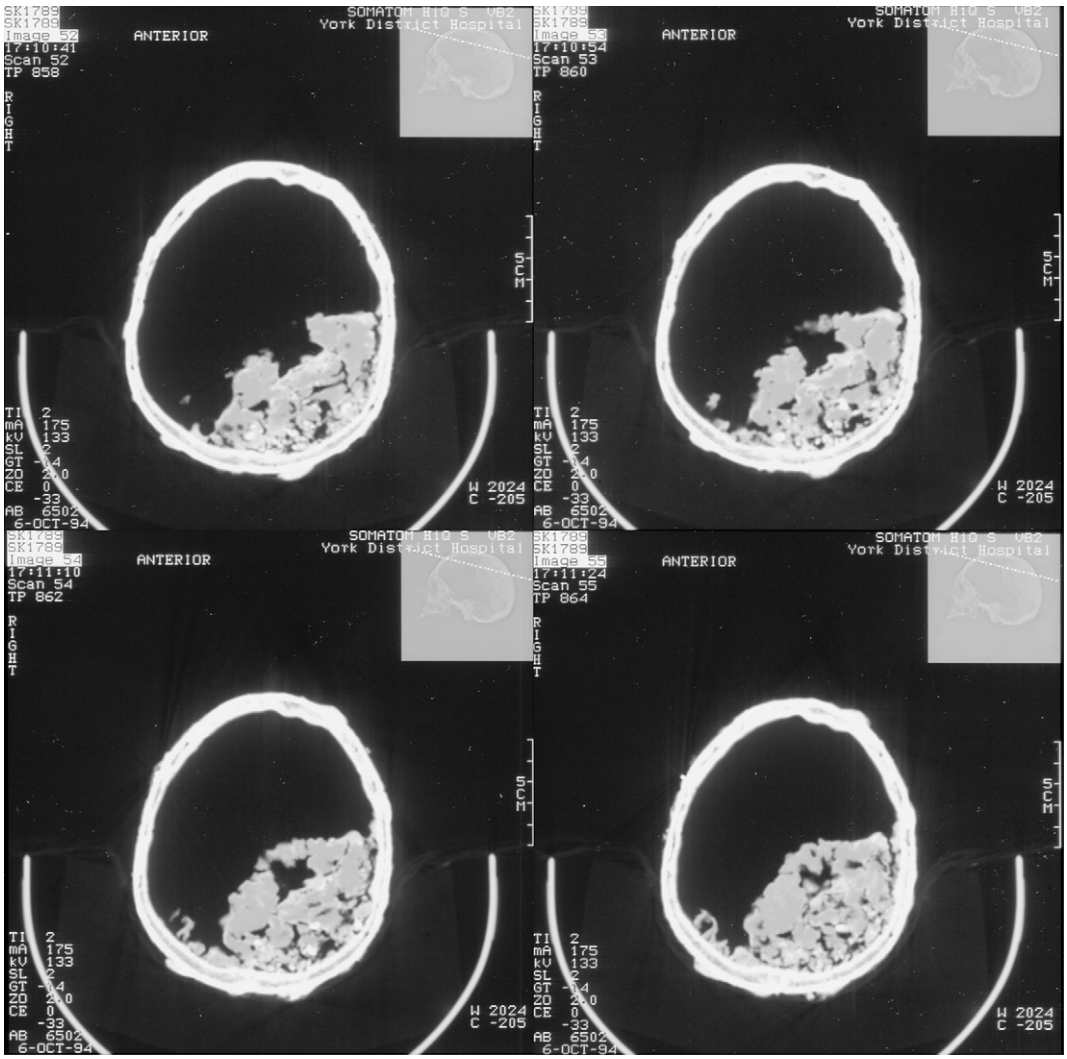

Fig. 14. Sequential CT images of a preserved brain in the skull of burial SK 2194 from the Humberside Archaeology Unit excavation at the Hull Magistrates' Court site, Kingston-upon-Hull (York Archaeological Trust).

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the state of putrefaction reached before this process was inhibited by equilibration with the burial environment. Differences in their histology and chemistry relate as much to their different states of preservation as to differences in the techniques applied to their investigation. Variation in the colours and textures of those that had subsequently dried out also fit Thouret's descriptions and again may reflect the extent of putrefaction that had occurred before preservation. Finally those brains that have not been stored in formalin, such as the Kingston-upon-Hull, Blackpool and Heslington examples, have proved quite stable for more than a year when kept hydrated in chilled storage, without physically deteriorating, developing an odour or apparently supporting fungal or bacterial growths. The persistent material that these brains have formed seems to be far more stable than fresh brain tissue: in that respect, it no more resembles fresh brain than casein plastic resembles the milk from which it was formed.

In the cemetery sites only a proportion of the burials produced recognisable remains of brains. Obviously not all the graves will have been suitably waterlogged and anoxic from the time of interment. However, observations of the distribution of other surviving organic materials (including textiles, leather and wood) at Kingston-upon-Hull indicate that even continuously waterlogged graves with intact skulls did not always produce brain tissue. Clearly factors other than the immediate burial environment must also influence the taphonomic trajectory of the brains. In one instance, Zihl, all the intact skulls contained very well preserved brain tissue, which in this unusual case can probably be directly associated with near-instantaneous death and burial (Pilleri and Schwab, 1970).

In attempting to understand the preservation of brains in skeletalised human remains, it is not helpful to conflate them with studies undertaken on brains from tanned bog bodies, frozen, desiccated, smoked or embalmed mummies and other instances where there has been significant additional soft tissue preservation. Brains discovered in skeletons from mass graves have not always been in typically wet or waterlogged sites (Brothwell and Gill-Robinson, 2002, 125; Radanov et al., 1992) but here the concentration of decaying bodies themselves may be creating the wet, anoxic conditions that seem to predispose preservation of the brain. The shrunken brain of a young adult female, from a 15th or 16th century burial in Yongin, Korea, investigated in 2005 (Kim et al., 2008), is very similar to the Heslington brain. Remains of the meninges (dura), blood vessels, grey and white matter, myelin structures and possible bacterial spores were observed and the soft tissue proved positive for both lipids and proteins. However the mechanism of preservation may be very different to the other cases reported here as hair was also preserved and there was soft tissue in the orbits and skin on the skull. Yongon is an example of a type of highly regulated burial practice in which the corpse was interred in a double wooden coffin, incorporating a layer of carbon, which was then thickly encapsulated in a lime–soil mixture. Korean tombs prepared in this way often lead to complete mummification where the sealing of the coffin remains intact and frequently brains still survive even where little other soft tissue is preserved. Kim et al. (2008) suggest that the lime–soil mixture may be involved in this process. From their account it is not clear how wet the burials are but the fibrous appearance of the wood suggests that it has degraded in a wet, alkaline environment.

Even brains recovered from skeletonised bodies in submerged marine environments, such as the 8 strong crew of the American civil war submarine HL Hunley (Jamie Downs pers. comm.) may have become preserved in a subtly different way. These brains and those from a few individuals who went down with the Tudor war ship, the Mary Rose, were similar to the terrestrial examples in colour and texture. However the Mary Rose examples 'liquified within a matter of minutes' unless immediately submerged in alcohol (Allen and Elkerton, 2005). Although many of the terrestrial examples were relatively quickly fixed in formalin, none had started to liquefy and several examples, including Heslington, have survived unfixed in chilled storage for extended periods. The dissolution reported for the Mary Rose specimens suggests that these have not formed the same persistent material.

5.4. Suggested mechanisms for preservation

Despite a lack of chemical evidence or other soft tissues, Haneveld (1984) speculates that the preservation of the Dordrecht brains is due to long-term submersion in a cold slightly acid tanning environment. Others have speculated that alkaline burial conditions may be involved (Doran et al., 1986; Tkocz et al., 1979). In the case of Warm Water Springs, Royal and Clark (1960) suggest that the ground water or sediments might have antibiotic properties, as stalagmite evidence indicates that the surviving brain mass was deposited before the site was inundated. However, as evidenced by the other examples cited here, the sediments probably only needed to have been wet and anoxic for preservation to have occurred in the intervening period. The most commonly held hypothesis is that the brains have become adipocere (Oakley, 1960; Papageorgopoulou et al., 2010; Tkocz et al., 1979), making it necessary to discuss this compound and its formation in some detail.

Limited analysis of preserved brain from the Paris cemetery also led Thouret (1791) to link the waxy material of the brain with adipocere. Today the term is strictly defined as the persistent waxy, complex formed by the hydrolysis and hydrogenation of adipose tissues during decomposition. This is a bacterially-mediated process that is most frequently associated with immersion in water or burial in waterlogged ground though moisture and bacteria intrinsic to the decomposing tissue can be sufficient to produce adipocere. Its formation inhibits further post-mortem changes and can make a corpse almost completely resistant to decay. Under favourable burial conditions considerable development of adipocere can occur within 30 days of interment (Fiedler and Graw, 2003). Observations of post World War II exhumations showed a direct correlation between body fat deposits and adipocere distribution (Mant, 1987). Adipocere was more localised to the cheeks, buttocks and abdomen in men than in women, reflecting in general their different patterns of subcutaneous fat distribution, and was more or less absent from the skeletons of emaciated concentration camp victims. Burial of a fully clothed body, directly into the soil, seemed to have produced the most rapid formation of adipocere.

The major lipid constituents (90–98%: Albright and Stern, 1998) of adipose tissue are triacylglycerols (TAGs) and these are broken down to form glycerol and mostly even-C number saturated fatty acids. As the formation of adipocere proceeds, the unsaturated fats in the adipose tissue are depleted, the proportion of palmitic, stearic and myristic acids increases and hydroxystearic acid is formed. Although commonly soft, greasy and white, adipocere can be reddish brown when fresh, become grey in older deposits, and develop a dry and brittle texture (Garland and Janaway, 1989). These differences in physical properties are due partly to cation exchange during different stages of formation of the adipocere, producing different salts of fatty acids. In the early stages of decomposition there is initially an abundance of sodium and then potassium salts as tissue cell structures disintegrate, but later, salts from the mineral components of the soil predominate. Potassium salts produce the wax-like deposits and sodium salts form a harder crumbly material (Vane and Trick, 2005). In the later stages of decomposition a quite brittle adipocere can be formed where calcium and magnesium salts are readily available (Forbes et al., 2005a).

Experimental work on adipocere formation by Forbes et al. (2005a,b,c) confirmed that the ideal factors for adipocere formation

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are the presence of moisture, bacteria, anaerobic burial conditions and a mildly alkaline pH. Adipocere formed readily between approximately pH 5–9 but was greatly curtailed in soils doctored to be highly acid (approx. pH 2.4) or highly alkaline (approx. pH 12.6). Other factors inhibiting formation included low temperature, aerobic conditions and sterilised soil. These results are consistent with the pivotal role of soil anaerobic bacteria in adipocere formation as their activity is curtailed in extremes of pH, low temperatures and aerobic conditions. Otherwise soil type had little effect on the rate of adipocere formation except that it was reduced in clayey soil (Forbes et al., 2005a).

Once formed, adipocere can be very resistant if the environment does not change. Fiedler et al. (2009) report a burial in which adipocere was still found after 1600 years even though the water table was thought to have varied considerably. However it is not the final product of decomposition and will itself decay over time. The half-life of adipocere in anaerobic conditions has been experimentally determined to be between 11 and 82 years: in air this is reduced 10-fold, to as little as 0.7 years (Fründ and Schoenen, 2009). Oxidation and decay by aerobic bacteria appear to be the main factors in this increased rate of deterioration (Fiedler and Graw, 2003).

As a mechanism for the preservation of brains from wet burial environments the formation of adipocere has several shortcomings. If the brains are preserved by adipocere, why is evidence of other soft tissue preservation almost entirely absent? Those brain remains not immediately placed in formalin did not deteriorate with the speed expected of fresh brain or of adipocere, but proved remarkably stable in dark, wet and chilled conditions. In addition, concomitant with the conversion of adipose tissue to adipocere is an increase in volume, sufficient to hide wounds or perforations the size of bullet holes (Mant, 1987). All of the brains in the literature cited, despite remaining hydrated, had shrunken substantially. Thouret (1790) observed that even in the Parisian mass graves (anything from 12 to 1500 individuals) where bodies were completely enveloped in a solid mass of adipocere, the preserved brains only filled a quarter to a third of the cranial cavity. Finally, although fresh brain tissue does contain ample lipids these are mostly of phospholipids and glycolipids. TAGs and free fatty acids account for only a few percent of the total lipid content and are probably associated with the blood and blood vessels, rather than the neural tissue (Suzuki, 1972). It is, therefore, highly unlikely that wet-preserved brains become adipocere.

It is possible, however, that adipocere is part of the mechanism of preservation. It was once thought that tissues such as muscle became adipocere (adiponeogenesis) but it has since been shown that the adipocere is formed from the liquified fatty acids that penetrate the decomposing muscle tissue under the pressure of gases formed during putrefaction (Fiedler and Graw, 2003). As a result, adipocere can form within tissues and organs with little intrinsic fat content and the state of preservation of the tissue will depend on how decayed it was at the time the adipocere formed. In this way, soft tissues, eyes, genital organs and internal organs, including the brain, can be preserved by adipocere (Fiedler and Graw, 2003). In all of these cases of wet-preserved brains, adipocere has been positively identified only in the Quimper-Bretagne brain (Papageorgopoulou et al., 2010). As the analysis was qualitative it could easily have been a minor constituent but its presence may have helped inhibit normal deterioration whilst more persistent material formed. It is possible that the red/brown deposit found on the surface of this specimen was adipocere but this was not analysed. In contrast, no adipocere was detected in the Heslington brain, probably because it was separated from the body, which indicates that adipocere within the brain tissue is not essential to the formation of a persistent mass.

The analytical study of the Heslington brain so far shows that, although chemical breakdown of proteins and lipids has been initiated, significant quantities of protein-derived and lipid-derived material have been preserved. The preserved material retains a higher proportion of hydrophobic components (amino acids in the case of proteins and high molecular weight hydrocarbon-like material in the case of the lipids) than fresh brain matter. It is not possible at the present time to determine if the hydrophobicity reflects the selective preservation of constituents that are not amenable to degradation or if it has acted in some way as a physico-chemical barrier preventing bacterial and fungal attack. The chemical analytical techniques applied to the other brains cited would not have been capable of detecting the high molecular weight material and its possible role in preservation is undergoing further investigation. These further studies include this brain and comparative modern materials.

6. Conclusions

All the published examples of wet preservation cited involved some level of investigation of the brain remains to evaluate surviving histology and the nature of the persistent material that had formed. Mostly they were regarded in isolation with little attention paid to the condition of the skeletal remains or detail of their burial environment. No attempt has been made to understand them as a group, to look systematically for common factors that might have led to their preservation or to see them, in an archaeological context, as evidence of the processes of death and burial. Based on the experience gained from the Hull Magistrates' Court excavation, the Heslington Brain Project has taken a more holistic view and its findings challenge many of the assumptions made in the past, such as the mechanism of preservation and their status as rare 'curiosities' rather than significant archaeological evidence.

Although the chemistry of the Heslington brain requires further investigation, it is clear that adipocere is not a pre-requisite in these cases. The literature yields a surprising number of instances in which brain is the only surviving soft tissue associated with otherwise skeletalised remains. In only one of these does there appear to be evidence for the presence of adipocere (Papageorgopoulou et al., 2010). The simplest explanation that accounts for all of the cases would seem to be that the circumstances of death and burial that predispose the formation of adipocere may also predispose the formation of a stable brain mass, but that neither process drives or necessitates the other. Burial immediately following death can only be inferred with certainty for the Zihl assemblage, for which we have proposed that the exceptional histological preservation may be a consequence of that rapid burial. If so, and if other means of determining the post-mortem, pre-burial interval can be developed, it may be possible to infer important details of the circumstances of death and burial of more human remains based on the survival and state of preservation of these remarkable ancient brains.

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specific protein samples, Bill Christie and Anna Nicolaou (University of Bradford) for their help in identifying the 2-hydroxyfatty acids and Michael Fagan (Hull-York Medical School) for testing the practicality of micro-CT examination.

Appendix 1

Records of brain masses in skeletalised human remains since 1960.

This catalogue concisely summarises the following information: location, date, number, context, description, investigative methods, further comments, and source.

Droitwich, U.K. Romano-British; 1 specimen female 44–55 yrs old; burial in wooden coffin at 3 m depth; small pieces of brain, shrunken, visible surface convolutions; unspecified chemical tests indicated replacement by 'wax', and noted substantial proportion of clay; identified as adipocere by Prof H. Spatz, but his reasoning is not given; (Oakley, 1960).

Florida, Warm Mineral Springs, USA. 8000 ± 200BC; 1 brain from 7 individuals; under 'several feet of soft sediment' containing leaves and wood in flooded, shallow limestone cave; pieces of soft, white brain mass with macroscopic structure; histological staining, ashing, unspecified chemical analysis; brain mass discoloured and shrank, possibly due to immersion in formalin; (Royal and Clark, 1960).

Florida, Little Salt Spring, U.S.A. 6860 ± 110BP; 1 brain from an unknown number of individuals excavated from cemetery thought to contain over 1000 inhumations; hard water, anaerobic, buried in peat with well-preserved wooden objects and plant remains in a wet, 'muck-filled' hollow leading to a sinkhole; 'substantial portion' of brain, surface convolutions, 'cellular processes', 'macroscopically well-preserved neural tissue'; aDNA; (Clausen et al., 1979; Pääbo et al., 1988).

Florida, Windover, USA. *Ca.* 7500BP; 91 brains from 168 individuals; burials in peaty sediments in swampy pond; shrunken to ¼ original size, tan-grey, soft, granular, fragile, some recognisable gross anatomy; CT, MRI, histological sections, TEM, aDNA; noted myelin traces; (Doran et al., 1986; Glen Doran pers. comm. 2008).

Zihl Canal, Coraux, Switzerland. La Têne; 18 brains from 18 individuals; under remains of wooden bridge in catastrophic flood debris at 6 m depth; greyish-white, 'humid', surface convolutions, reduced in volume; histological section; axons and possible fungal structures identified; (Pilleri and Schwab, 1970).

Svenborg, **Funen**, Denmark. Medieval; 56 brains from 74 individuals; Franciscan cemetery, graves cut into alkaline clays frequently flooded; preservation varied but not in relation to presence of coffin, dark grey, soft, 'soapy', shrunken to ½ size; fixed in formalin, histological sections, SEM, 'qualitative' chemical analysis unspecified; traces of axons noted, cholesterol and phospholipids detected; (Tkocz et al., 1979).

Dordrecht, Netherlands. 14th century; number unknown; Minorites monastic cemetery; 'well preserved' with visible convolutions; fixed in formalin, CT, MRI, histological sections, TEM; CT confirmed ventricles and post-mortem cavities, staining confirmed neurons, cell fibrillary structures, vague contours of vascular structures, lipids and myelin not detected, no fungi detected; (Haneveld, 1984).

Sandwell, U.K. 14th century; 1 brain from 1 individual; grave within Benedictine Priory, waterlogged, neutral; soft, black material within cranium; fixed in formalin, histological section, electron probe microanalysis, no organic analysis undertaken; traces of shroud material survived; (Flinn, 1989).

Kingston-upon-Hull, Magistrates' Court, U.K. Medieval; 25 brains from *ca* 250 individuals; Augustinian Friary cemetery inside and outside buildings, waterlogged; grey-brown, soft, slightly resilient, shrunken, convolutions visible, one apparently liquefied then reformed as soft, granular endocast, becoming nodular and granular or black, glossy and resinous on drying in storage; CT, histological section, FT Raman spectroscopy; (O'Connor, 2002).

Scole, Norfolk, U.K. Roman; 1 brain from 1 individual; burial beneath wooden plank in alluvium, riverside, possible murder victim; granular endocast; (O'Connor, 2002; G. Turner-Walker pers. comm.).

Aalborg, Denmark. Medieval; 10 brains from an unknown number of individuals; Franciscan friary cemetery; varying states of preservation; (O'Connor, 2002; J. Nielson in litt.).

Salisbury, U.K. Medieval; 1 brain from an unknown number of individuals; Friary burial; identified as a fungal pseudomorph by a morbid anatomist, no analyses; (O'Connor, 2002; M. Corfield pers. comm.).

Norwich, U.K. 1 brain; context and date uncertain; (O'Connor, 2002; B. Ayers pers comm.).

Quimper-Bretagne, France. AD1250–1275; 1 brain from 1 individual, 18-month-old child; skeletonised remains wrapped in leather within wooden coffin, burial in wet clay; greyish, compact, convoluted surface, traces of meninges, harder, darker medial surface may be more degraded; fixed in formalin, CT, MRI, histological section, aDNA, GC–MS; Nissl bodies identified, diverse fatty acids detected, poor DNA preservation (formalin); (Papageorgopoulou et al., 2010).

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