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A conscious rethink: Why is brain tissue commonly preserved in the archaeological record? Commentary on: Petrone P, Pucci P, Niola M, et al. Heat-induced brain vitrification from the Vesuvius eruption in C.E. 79. N Engl J Med 2020;382:383-4. DOI: 10.1056/NEJMc1909867

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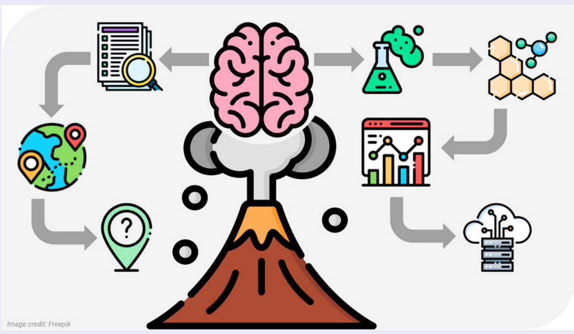
ABSTRACT
Brain tissue is ubiquitous in the archaeological record. Multiple, independent studies report the finding of black, resinous or shiny brain tissue, and Petrone et al. [2020 "Heat-induced Brain Vitrification from the Vesuvius Eruption in C.E. 79." *N Engl J Med*. 382: 383–384; doi:10.1056/NEJMc1909867] raise the intriguing prospect of a role for vitrification in the preservation of ancient biomolecules. However, Petrone et al. (2020) have not made their raw data available, and no detailed laboratory or analytical methodology is offered. Issues of contamination and misinterpretation hampered a decade of research in biomolecular archaeology, such that addressing these sources of bias and facilitating validation of specious findings has become both routine and of paramount importance in the discipline. We argue that the evidence they present does not support their conclusion of heat-induced vitrification of human brain tissue, and that future studies should share palaeoproteomic data in an open access repository to facilitate comparative analysis of the recovery of ancient proteins and patterns of their degradation.



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Introduction

> And indeed, the rest of the heads buried there were completely dried up; however, one brain within the skull was discovered many years after burial, still soft and wet and free from decay, even when exposed to the light of day. (Raynaud 1651, translated by A. Morton-Hayward)

As the old saying goes, you wait an age for a bus and then two come along at once: Petrone et al.'s (2020) report on the recovery of "vitrified" human brain tissue from Roman Herculaneum is published only two weeks after Petzold et al.'s (2020) report on the recovery of an extensive brain proteome from a human brain from Iron Age Yorkshire. Petrone et al.'s (2020) claim that brains "are rare archaeological discoveries" may not seem contentious, given that brain decomposition post-mortem is rapid (Hayman and Oxenham 2017; Table 3). Yet remarkably, the statement is false. The true curiosity lies in why the brain seems to be the most

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commonly preserved soft tissue in ancient human remains and, moreover, why it preserves such an extensive proteome (Petzold et al. 2020).

The earliest published report of the finding of preserved brain tissue appears to be that of the French theologian Père Théophile Raynaud (1651), who describes a brain, buried for 25 years in a grave in Avignon, apparently undecomposed and astonishingly preserved. Further reports in the seventeenth (Garmann 1660; Herbinius 1675) and eighteenth centuries (Thouret 1790; Fourcroy 1791; Thouret 1791; Fourcroy 1793) led Professor Elliot Smith of the University of Cambridge and Cairo Medical School, working in cemeteries throughout ancient Egypt, to recognise that preservation of the brain was far from a singular circumstance, lamenting that his colleagues "seem to be not only ignorant of this fact, but even deny the possibility of its occurrence" (Elliot Smith 1902). Indeed, multiple studies report the preservation of black, resinous or shiny brain tissue in the archaeological record (Table 1), and the "remnant liquid or paste" found in modern crania from forensic contexts (Hayman and Oxenham 2017; Table 3) echoes the description of resinous-like, organic material reported pooled in the crania of many ancient mummified corpses (e.g. Hawass and Saleem 2011; Proefke et al. 1992; Rühli, Chhem, and Böni 2004; Lynnerup 2010; Wade et al. 2010; Saleem and Hawass 2013).

Vitrification?

Both the sheer abundance of preserved brain tissue as well as its frequent presence in otherwise skeletonised individuals (that is, in the absence of other soft tissue preservation) demands greater attention. Petrone et al. (2020) helpfully introduce the concept of vitrified organic material to the study of ancient tissues. Contemporary understanding of vitrification is underpinned by studies of anhydrobiosis (Crowe, Carpenter, and Crowe 1998; Rebecchi, Altiero, and Guidetti 2007), a very low-energy metabolic state that enables organisms to persist in a condition of suspended animation. As proteins dehydrate, sugars typically replace the water; this science has been used to develop products that form glass in the presence of macromolecules (Slade and Levine 1995) and conceptually the notion offers scope for exploring the preservation of ancient proteins (c.f. Chang and Pikal 2009).

Unhelpfully, Petrone et al. choose a very narrow definition of vitrification: "tissue that has been burned at high heat and turned into glass or a glaze" (2020). On the basis of the limited evidence presented in their paper, we cannot discount the idea that proteins are vitrified (*sensu stricto*), but we are less persuaded that they have evidenced the role of heat.

Evidence for high temperature?

We accept that the pyroclastic flows from Vesuvius are of such high temperatures that they would have burnt wood, and Petrone et al. (2020) demonstrate heat-induced vitrification of a wood fragment of a charred beam from a workshop situated in the third Cardo, nearby the Collegium Augustalium. However, this evidence does not imply a causal relationship to the vitrification process observed in the brain. While charcoal analysis is common practice in cremation studies and usefully informs discussions on, for example, pyre efficiency and structures, funerary processes and the temperature of burning (e.g. O'Donnell 2016; Ortiz, Ramos, and Alavar 2017), substantially larger sample sizes are generally used than those analysed in this paper. Experimental work by McParland et al. (2010) concluded that "vitrification of charcoals is not a function of high temperature" and further that "when subjected to high temperatures (up to 1100°C) in the laboratory, the charcoals … did not show characteristics diagnostic of vitrification" (McParland et al. 2010). Moreover, previous charcoal analysis at Herculaneum itself has concluded that wooden structures were burned to between 240-370°C (Caricchi et al. 2014), lower than the temperature estimates suggested by Petrone and colleagues (2020). It is difficult to examine the temperature prediction work here, since taxonomic confirmation of the species of the charcoal fragment has not been provided, nor have the subsequent reflectance calibration curves.

However, we have recently reported on the temperature estimates of human remains from this site (Martyn et al. 2020). In our work we utilised the transformative relationship between the crystal structure of bone and heat to determine that the deceased had experienced low temperatures in comparison to cremation funerary practices, in which temperatures can reach over 900°C. Note that our individuals were located in the beachfront fornici, and thus sheltered and buffered in a manner different to the Petrone et al. (2020) example. This method of predicting the temperature of burning from skeletal remains is now well-established, having been used in a variety of archaeological cremation contexts; including the Roman period (Thompson et al. 2016). It is based on the robust, curvi-linear relationship that exists between temperature intensity and crystallinity measures, with values derived from Fourier transform infrared (FTIR) spectroscopy analysis of the osteological material (Thompson 2015; Ellingham, Thompson, and Islam 2016; Thompson et al. 2016; Marques et al. 2018). Given Petrone et al.'s assertion of "extreme radiant heat … able to ignite body fat and vaporize soft tissues" (2020), we argue that the aforementioned, routine method should have been applied to this individual in order to ascertain whether a high temperature was in fact achieved.

Table 1. Reports of vitreous/resinous and/or black preserved brain tissue.

Description	Context; Location (Period)	Ref.
"In a letter to Virchow, dated Cairo, February 21, 1897, Fouquet mentioned finding resinous material in a skull at El Omra" (Lamb 1901)	Burial in dry soil; Al Omrah, Upper Egypt (prehistoric, c. 4400–3500 BC)	(Lamb 1901)
"Salkowski reported to the Berlin Anthropological Society [in 1897] the results of his most exhaustive examinations of the contents of some Egyptian mummy skulls … The masses were found to be usually dark brown, were somewhat friable, and broke with a shining fracture: he obtained from them an alkaline ash, salts of phosphoric acid, resinous matter, fatty acids, and neutral fats which always gave a strong reaction of cholesterin. His conclusions were that in some cases brain matter was probably present, in others its presence was doubtful; from which Virchow was moved to question whether the material was actually brain or merely embalming material." (Lamb 1901)	Burial in dry soil; Al Omrah, Upper Egypt (prehistoric, c. 4400–3500 BC)	(Lamb 1901)
"The masses may be black or dark brown, breaking with a shining fracture, or they may be of a much lighter brown colour, or even a light grey, and present a wood-like texture. The latter variety often have patches of white substance on their surface." (Elliot Smith 1902)	Burial in dry soil; Ancient Egypt (prehistoric to Coptic Period, c. 4400 BC–1st C. AD)	(Elliot Smith 1902)
"heat-affected brains as almost bioporcellain specimens" (Altinoz et al. 2014); "carbonized tissue samples consisting of brain tissue were highly fragile" (Altinoz et al. 2014)	Fire-affected tumulus; Kütahya, Western Anatolia (Bronze Age, c. 1900–2000 BC)	(Altinoz et al. 2014)
"The brain itself is usually found either as a loose, shapeless, somewhat flattened mass, or as smaller masses adherent to the several intracranial fossae, or both. The color varies from light brown to nearly black; it has the consistence, toughness, and brittleness of ordinary resin; in its center is sometimes found a whitish, waxlike substance. The mass usually burns with a dull, smoky flame, like resin, with a blackish residue … [In 1857] Professor Vogel, of the University of Giessen, examined many of these masses, and reported that chemical and microscopical analyses showed them to contain brain fat and dried blood cells, with no foreign substance. Dr W. M. Gray of the Army Medical Museum at Washington, has also examined these masses microscopically and reports that they dissolve readily in caustic potash solution and are composed of numerous cells varying in shape and size, mixed with unrecognizable granular material, with an occasional small mass of blackish pigment; macroscopically they break like wax and have a greasy feel. Salkowski also examined the skull contents in one case; they consisted of a soft, brownish, friable mass mixed with some sand, and burned with a bright flame and the odor of fat and burning horn. He obtained a fatty mass by extraction with alcohol and also a strong reaction of phosphoric acid, from which he concluded that it was undoubtedly brain substance." (Lamb 1901)	Mummification; South America (Incan Empire, c. 15th–16th C. AD)	(Lamb 1901)
"Color, dark brown, approaching black externally; a lighter brown or tan color where the outer part is chipped away; the appearance is everywhere granular; in one or two places where the outer part has been fractured, black, glistening surfaces appear beneath. Scattered in crevices in the general surface is a small quantity of a whitish powder. All the surfaces are convoluted and the general appearance is that of a brain … Some cells contained a black or dark brown pigment" (Lamb 1901)	Burial in ash and clay; Ohio, U.S.A. (pre-1670 AD)	(Fowke and Moorehead 1894; Lamb 1901)
"The dehydrated masses were very light in weight and brittle like furnace clinker. The surface colours of reddish orange and black still predominated but splashes of yellow, black veining and dustings of cream and yellow powder [were observed] … The most dehydrated nodules snapped to reveal a black, often glossy interior with a rippled fracture surface reminiscent of a hard resin" (O'Connor 2002)	Augustinian friary cemetery; Kingston-upon-Hull, UK (c. 1316–1539 AD)	(O'Connor 2002)
"the favorable condition of a dry soil, has preserved a portion of the brain mass with its membranes in the form of a hard dark ball." (Putnam 1888; see also Lamb 1901)	Burial of isolated cranium; Massachusetts, U.S.A. (c. 17th C. AD)	(Putnam 1888)
"j'en ai trouvé des masses très-petites, entièrement noirâtres à l'extérieur … [avec une] grand dureté … Ces masses, toutefois lorsqu'elles étoient séchées & exposées à l'air, paroissoient être indestructibles." (Thouret 1791) Translation by A. Morton-Hayward: "I have found very small masses entirely blackened on the surface … [with a] great hardness … However, whenever these masses were dried and exposed to the air they seemed to be indestructible."	Charnel house; Paris, France (c. 18th C. AD)	(Thouret 1791)
"The preserved structures strongly resembled human brains, although they were hard in consistency and black in color" (Radanov et al. 1992); "suitable temperature and ventilation apparently enabled rapid evaporation of intracellular brain fluid" (Radanov et al. 1992)	Mass grave; Dobrinishte, Bulgaria (c. 1942–1947 AD)	(Radanov et al. 1992)
Black but not vitreous/resinous		
"black material" (Melton et al. 2010)	Log-coffin burial; Gristhorpe, UK (Early Bronze Age, c. 2000 BC)	(Melton et al. 2010)
"One of the largest [brain] masses had an area of black membranous material, perhaps a fragment of the meninges" (O'Connor et al. 2011); "the brain itself and … the black sludge occupying the cavity between the brain and the cranium" (O'Connor, Edwards, and Ali 2016)	Waterlogged pit; York, UK (c 673–482 BC)	(O'Connor et al. 2011; O'Connor, Edwards, and Ali 2016)
"brownish-black superficial discolouration observed on the left parietal lobe" (Serrulla et al. 2016)	Mass grave; Burgos, Spain (1936 AD)	(Serrulla et al. 2016)

Relatedly, the description of the remains provided ("the skull and the postcranial bones are exploded and charred"; Petrone et al. 2020, Sup p. 6) does not support a high temperature event. There is strong disagreement that skulls explode as a result of extreme heat (Symes et al. 2014), and moreover charring is indicative of low- to medium-intensity burning events, since it demonstrates the presence of organic material within the bone (Ellingham, Thompson, and Islam 2016; Thompson et al. 2017; Wärmländer et al. 2019).

Lipid chemistry

Akin to many writers before them (Oakley 1960; Tkocz, Bytzer, and Bierring 1979; Karlik et al. 2007; Serrulla et al. 2016), Petrone et al. (2020) claim that when found, preserved brains are typically saponified. Like Serrulla and colleagues (2016), who studied 45 brains excavated from a Spanish Civil War mass grave, Petrone et al. (2020) report the presence of abundant free fatty acids, which Serrulla et al. (2016) cite as evidence of saponification, a process resulting in adipocere formation and an attendant increase in the volume of affected soft tissue (Mant 1987). Every preserved brain in the extant literature, however, is described as substantially reduced in volume, regularly to around a fifth that of fresh tissue (O'Connor et al. 2011). Similarly, whereas adipocere ("grave wax") is associated with either a hard, crumbly texture or a soft, pastelike consistency depending on ionic involvement (Vass 2001; Powers 2005), preserved brains have been described in the literature (O'Connor et al. 2011) with a broad gamut of different textures, such that this observed diversity cannot be explained by saponification (or any single mechanism) alone.

The lack of a detailed methodology outlining how the extracts were derivatised prior to GC-MS by Petrone et al. (2020) confounds evaluation of the data presented in Table S2. Nonetheless, no long chain ketones were identified, which would be expected through condensation of free fatty acids purportedly exposed to high temperatures (Evershed et al. 1995). While adipic and margaric acids may be minor metabolites, they are hardly diagnostic for hair or sebum as Petrone et al. (2020) appear to suggest, since they occur in a wide range of natural products. The C6 dicarboxylic acid (adipic acid) may be an oxidation product of longer-chain unsaturated fatty acids, or a contaminant; it is not a major fatty acid expected from hair or skin. Additionally, the Delplancke et al. (2018) study referred to by Petrone et al. (2020) demonstrates that these metabolites are found at significantly increased concentrations in the hair of pregnant women with gestational diabetes mellitus, rather than as major lipid components. The survival of fatty acids attributed to brain and hair seems inconsistent with temperatures sufficiently high for the vitrification of wood (482-524°C; Petrone et al. 2020, Sup Figure S5), at which temperatures not only are these fatty acids volatile, but also unstable (Milovanović et al. 2006; Li et al. 2018).

Protein identifiers and the human brain

Evidence for the palaeoproteomic data in the study was only provided as supplementary material in the form of a list of proteins (Petrone et al. 2020, Sup Table S1). Petrone et al. (2020) have not made their raw data available, no controls are listed, no uniquely identified peptides are reported and there are no references to how protein identifications were made or verified (Latterich 2006; Taylor et al. 2007). Seven named proteins were obtained from two samples: Q71F56, P04035, Q16864, Q9H1Z4, Q96ST2, Q2KJY2, P08708. The third column of Table S1 (headed "Organism"; Petrone et al. 2020 Table S1) is redundant, given that *Homo sapiens* is not the only species that expresses these proteins; the final column (headed "Expression") is equally misleading, appearing to suggest that these proteins are exclusively expressed in the brain regions listed. However, all are expressed in multiple tissues throughout the body, including skin (a common modern contaminant of ancient material; Hendy et al. 2018), and without strict controls the possibility cannot be excluded that these may have been introduced at any time during sample collection and analysis. Further, while Petrone et al. (2020) explicitly contend that the seven proteins identified in their study are "highly expressed in human brain tissues", none of these proteins match any of the 881 proteins recovered from the Heslington brain (https://www.ebi.ac.uk/pride/archive/; identifier PXD014178), nor the 41 abundant brain proteins from the Iceman's brain (Maixner et al. 2013; Table 1), although all were reported by Ping et al. (2018) from modern human brains.

Proteomic data from both the Heslington brain (Petzold et al. 2020) and that of the Iceman (Maixner et al. 2013) offer quantitative information, which is essential for statistical analyses addressing cross contamination and other sources of bias, as well as for empirical validation of spurious findings. Limited by being qualitative and ambiguous, we argue that the proteomic data as reported by Petrone et al. (2020) do not support their conclusion that the find unambiguously represents human brain tissue.

Future directions: integrating analysis and sharing data

Cremation studies have lagged behind those involving inhumed remains due to the additional challenges inherent to these particular contexts of death, such that discussion of the additional interpretative power preserved human remains provide both archaeologists and anthropologists in cremation contexts is welcomed.

Likewise, the near simultaneous publication of reports on preserved ancient brain tissue (Petrone et al. 2020; Petzold et al. 2020) calls attention to our current lack of understanding of the means by which neural tissues preserve in the archaeological record. In this respect, we welcome the study of Petrone et al. (2020), which both highlights the importance of combining proteomic and lipidomic investigation with analyses of associated skeletal material, and raises the intriguing prospect of a potential role for vitrification in the preservation of ancient biomolecules. Certainly, the vitrification process is compelling and brains mirroring this type of preservation have been reported previously (Table 1). By contrast, in the case of an Iron Age brain from Britain, Petzold et al. (2020) posit preservation by a process of protein aggregation, yielding the richest ancient proteome yet reported (>800 proteins) – a figure they believe to be an underestimate.

Yet despite the great promise of these avenues for future investigation, an unfortunate tendency to treat the preservation of brain tissue as a "unique" phenomenon (Clausen et al. 1979; Radanov et al. 1992; Gerszten and Martifínez 1995; Chudá, Dörnhöferová, and Marián 2010) has to-date dissuaded any attempt to arrive at an evidence-based consensus on the material's biochemical nature or its bioarchaeological value, let alone the potential mechanism(s) of its preservation. O'Connor and colleagues (2011) helpfully detailed over 200 preserved brains reported in the preceding 50 years; however, ongoing research by one of our authors (A. Morton-Hayward, unpublished data 2020) has uncovered a thousand more ancient brains in reports dating back to the seventeenth century. Indeed, there can be no doubt that brain tissue preserves in an unexpected, unappreciated and as-yet unexplained variety of depositional environments, and there is a clear need for comprehensive, systematic investigation of this intriguing material.

The ubiquity of ancient brains begs the question: *Why is neural tissue the most commonly preserved soft tissue in the archaeological record?*

It might be suggested that the skull affords the brain some manner of protection from exogenous decomposition variables (e.g. [in]vertebrate scavenging, humidity/aridity, soil pH, rainfall, etc.; Mann, Bass, and Meadows 1990), in similar fashion to bone marrow shielded within the medullary cavity, putrefaction of which appears to be inhibited where cortical integrity is maintained (Roll, Beham, and Beham-Schmid 2009; Cartiser et al. 2011). Indeed successful genomic typing of brains recovered from waterlogged environments, but not from the intact crania within which they were preserved (Graw, Weisser, and Lutz 2000), might be seen to support this notion. Conversely however, preserved brains have been discovered in skulls fragmented by both perimortem trauma and extensive weathering postmortem (Radanov et al. 1992; Eklektos, Dayal, and Manger 2006; Serrulla et al. 2016), which might alternatively suggest that under certain (as-yet unknown) conditions the brain itself, irrespective of any protective action of the skull, is relatively resilient to putrefaction.

One possible answer may be the architectural organisation of the tissue. In order to maintain its hundreds of trillions of synaptic connections, the human brain is reliant upon the structural stability conferred by a matrix of intermediate filaments (IFs), a group of protein polymers supporting neurons and axons (Petzold 2005; Khalil et al. 2018) as well as astrocytes (Lu et al. 2013; Petzold 2015). IFs are unusual proteins, possessing large polyanion tails and multiple phosphorylation sites, and being inherently unstructured and able to self-assemble into polymers; they are known to form both intra- and extra-cellular aggregates in pathological conditions (Dunker et al. 2001; Petzold et al. 2008; David et al. 2010; Petzold, Tozer, and Schmierer 2011; Babu, Kriwacki, and Pappu 2012; Jucker and Walker 2013). These key neural building blocks are also present in the peripheral nervous system, which we would be hesitant to dismiss (and is easily overlooked) as a potentially rich reservoir of palaeoproteomic data (c.f. Gerszten and Martifínez 1995; Kim et al. 2006).

Both the central and peripheral nervous systems require considerably more rapid information transmission than a single cell can provide, such that an intimate association between the protein-rich axon and the lipid-rich myelin sheath evolved in part to accommodate this demand for quick, saltatory conduction. Concomitantly, however, this neuroanatomical arrangement rendered the axon energetically and spatially isolated (Nave 2010). The high lipid content of the brain, surpassing that of any other organ, increases the likelihood of hydrophobic protein aggregate formation (Fink 1998) and is therefore not only quantitatively relevant, but likely also heterogeneously distributed. In this light it is worth noting that, while Petzold et al. (2020) observed an immune response from both ancient white and grey matter in the Heslington brain, the highest degree of immunogenicity was found for lipid-rich myelin.

Analytical strategies

Simple assays such as elemental composition, chiral amino acid analysis and pyrolysis-gas chromatography/mass spectrometry (e.g. Larter and Douglas 1980) might be usefully employed to facilitate an accurate and precise characterisation of diverse patterns of preservation and, when combined with genomic, proteomic and lipidomic strategies, enable exploration of the research potential of this commonest of preserved soft tissues.

Given that problems with contamination and misinterpretation disrupted almost a decade of research in the nascent field of biomolecular archaeology, data sharing has become critical for validity, reliability and replicability in the study of ancient biomolecules; such that in the case of palaeogenomics, almost 100% of published ancient DNA data are now regularly made available (Anagnostou et al. 2015). In the case of palaeoproteomics, questions concerning both how to avoid being misled by cross contamination and, concurrently, how to authenticate identifications rightly remain the focus of ongoing discussions, although several guidelines have already been proposed that detail present best practices (Schroeter et al. 2017; Hendy et al. 2018; Ramsøe et al. 2020). In light of these challenges – and since the controversy around the reported detection of dinosaur bone collagen (Schweitzer et al. 2007), which, in this instance, saw the raw data eventually made available to the wider scientific community – the need for the routine release of data has not only been recognised but has now become standard in the discipline. Moving forward, we echo Hendy et al. (2018) and recommend that future research shares data on ancient human brain proteins in an open access repository (accepted practice for the reporting of ancient human proteomes), enabling comparative analysis of the recovery of proteins and patterns of their degradation, which will in turn help to illuminate pathways of decay (Mackie et al. 2018; Ramsøe et al. 2020).

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