Journal of Archaeological Science 36 (2009) 35–42

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

Indications of embalming in Roman Greece by physical, chemical and histological analysis

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ARTICLE INFO

Article history:
Received 10 March 2008
Received in revised form 27 June 2008
Accepted 8 July 2008

Keywords:
Electron microscope
Energy dispersive X-ray analysis
Gas chromatography/mass spectrometry
Hair
Lead coffin
Mummy

ABSTRACT

The partially mummified remains of a high-status female (ca. 1700 BP, Thessaloniki, Greece) were found inside a Roman-type marble sarcophagus containing a lead coffin. The individual was positioned on a wooden pallet, wrapped in bandages, and covered with a gold-embroidered purple silk cloth. Besides the clothes, remnants of soft tissue as well as the individual's original hair style and eyebrows were exceptionally well preserved. In addition to the macroscopic examination, microscopic and biochemical analyses were undertaken. Scanning electron microscopy (SEM), energy-dispersive X-ray (EDX) analysis, and gas chromatography/mass spectrometry (GC/MS) were applied to examine the tissue preservation and probable mechanisms of mummification. The presence of chemical components, such as sesquiterpenes, triterpenoids, and diterpenoids, originating from coniferous and pistacia resins, myrrh, and other spices, verify ancient information on preparation methods of the dead in Greek and Roman times. These chemical components are thought to have played a prominent role in the mummification mechanism in this particular case. The potential effect of the lead coffin in the mummification process was also examined. Energy-dispersive X-ray analysis failed to detect lead penetration into the tissues, suggesting that the coffin played a limited role in the preservation of soft tissue.

1. Introduction

The study of human skeletal remains and burial customs provides important information concerning the demography, nutrition, and health status of past populations; they may also provide data about social identity, social organisation, ideology, rituals, religion, and symbolism (Larsen, 1997; Pearson, 1999; Walker, 2000). Treatment of the corpse after death varied considerably in different periods and regions; the most popular burial practises worldwide included inhumation, cremation, and mummification (Pearson, 1999). Due to the soft tissue preservation, mummified bodies may represent an even more detailed source of information concerning mortuary practices and burial rites (Lynnerup, 2007).

Soft-tissue survival on prehistoric bodies, however, is rare and, when present, is usually due to multiple mummification mechanisms that can be either anthropogenic (artificial), spontaneous (natural), spontaneous-enhanced, or indeterminate (for term definitions, see Aufderheide, 2003; Lynnerup, 2007). Mummified human bodies or partially mummified human tissues have been found sporadically in every part of the world (Cockburn, 1980; Lynnerup, 2007).

In the present study, we report on a rare case of a partially mummified body dating to 1700 BP found in Northern Greece. Mummified human soft tissue and hair are uncommon for this period in Greece, and no histological or chemical study of preserved human tissue has ever been made of a Greek mummy. The present work seeks to describe this rare finding and to determine the cause of this unusual preservation.

2. Materials and methods

An individual was found inside a Roman marble sarcophagus containing a lead coffin. The marble sarcophagus, measuring 95 cm × 200 cm, was uncovered in 1962 during archaeological excavations on the eastern cemetery of Thessaloniki (Northern Greece), which was used from the Hellenistic to the Byzantine Periods for burial and ritual practises (Lambrothanassi, personal communication, 2006). The individual was positioned on a wooden pallet in the interior of the lead coffin and covered with

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doi:10.1016/j.jas.2008.07.003

a gold-embroidered, purple silk cloth (Kampassakali and Varella, 2002; Kampassakali et al., 2003). The body was wrapped with cotton or linen bandages, some of which were found *in situ*, especially around the upper and the lower limbs.

The skeleton, remnants of soft tissues especially on the axial skeleton, hair, and loose compact particles varying from 3 to 12 cm long were found inside the lead coffin. The compact particles are composed of several layers, probably a combination of cloth and soft tissue, and have a dark brownish colour. Some of them are extremely fragile, being of powdery texture.

Prior to any microscopic analyses, the gross anatomy of the skeleton and the soft tissues were examined macroscopically for paleopathological lesions. Sex determination was based on morphological features of the skull, the pelvis (Workshop of European Anthropologists, 1980), and the long bones (Bass, 1995). Age estimation was made according to the degree of closure of the cranial suture (Meindl and Lovejoy, 1985), the changes of the pubic symphyses (Suchey et al., 1984; Brooks and Suchey, 1990), the auricular surface of the pelvis (Lovejoy et al., 1985, Meindl and Lovejoy, 1989), and the degree of dental attrition (Brothwell, 1981; Miles, 1962). The stature was estimated based on the measurements of long bones (Bach, 1965).

Standard histological analysis of the hair and the compact particles found within the lead coffin was performed. The samples were fixed in 4% formalin and immersed in multiple baths of progressively more concentrated ethanol to dehydrate the tissue; xylene was used as a clearing agent. The tissues were embedded in paraffin wax and sectioned into ultra-thin sections (5–8 μm), using a rotation microtome (Microm HM 325, Adamas Instrumenten, Rhenen, Netherlands); for half the samples routine hematoxylin and eosin (HE) staining was used. A wide-field microscope (Zeiss Axiophot, Carl Zeiss AG, Feldbach, Switzerland) equipped with a digital camera and a confocal laser-scanning microscope (Leica SP2, Leica Microsystems GmbH, Wetzlar, Germany) were used for the analysis of the sections.

In order to describe the fine structure of the soft tissue and scalp hair a scanning electron microscopy analysis was performed. Hair shafts from the individual's scalp hair and the distal epiphysis of a second right metacarpal were sputter-coated with gold palladium (15 nm) and examined with a scanning electron microscope (JEOL JSM-6360LV, JEOL Ltd. Tokyo, Japan).

A part (17 mg) of the compact, powder-like, particles, a proximal hand phalanx (1.194 g) with soft tissues on it, and two samples of scalp hair (107.5 mg and 86.5 mg) were further analyzed chemically. All samples were extracted in a non-destructive manner, first with methanol in an ultrasonic bath for 30 min, then without ultrasonification for 2 days, and finally with *t*-butyl methyl ether for 30 min again in an ultrasonic bath. Insoluble parts were removed by centrifugation. The combined extracts were analyzed by gas chromatography/mass spectroscopy (GC/MS, Varian Saturn 4D).

Additional elemental analysis was performed using an energy-dispersive X-ray analysis (EDX). Bone particles with adhering soft tissues on them from a third left metacarpal and a proximal hand phalanx were examined. The analysis was performed in the Laboratory for Electron Microscopy and Nano-Analytic (EMOTT AG, c/o University of Zurich) and the Microscopy Centre (University of Zurich). The samples were investigated in the first laboratory, without any further preparation, in an environmental scanning electron microscope (Philips, ESEM Xl40) equipped with an energy-dispersive X-ray-system (EDAX Sappire); in the second laboratory, the samples were sputter-coated with carbon and analysed using a scanning electron microscope (Zeiss Supra 50 VP, Carl Zeiss MicroImaging GmbH, Offenbach, Germany).

3. Results

3.1. Skeleton

According to the sex and age determinations, the skeleton belonged to a mature female individual between 50 and 60 years of age, with a stature of 160 cm.

The individual had lost ante-mortem three mandibular teeth: the right second premolar and molar, and the left first molar. Slight calculus deposits on the mandibular anterior teeth, slight resorption of the alveolar crest, and an infraosseus periodontal pocket on the right upper incisor and on the left and right central lower incisors were further recorded. Severe attrition was present throughout the entire dentition, especially on the anterior upper and lower teeth. The upper central and lateral incisor displayed characteristic U-shape attrition.

Slight signs of degenerative lesions were observed on the articular facets of the first and the second cervical vertebrae, on the body of the fifth and sixth cervical vertebrae, and on the articular facets of the lumbar vertebrae. Similar findings were observed on the sternal epiphysis of both clavicles, on the right scapula (glenoid fossa) and on the pelvis (right and left auricular surface).

3.2. Hair

The hair was detached from the scalp (Fig. 1a) although sporadic hair shafts were still present on the right and left parietal bones. The eyebrows were preserved in excellent condition. The current colour of the hair was brown with reddish highlights.

Scanning electron analysis of the scalp hair showed that the cuticle was very well preserved in many hair shafts; the cuticular cells were observable as overlapping scales with irregular edges (Fig. 1b) very similar to modern scalp hair that has not undergone any degradation process (Fig. 1c). Although the hair shafts showed the absence of cuticular scales there were limited signs of other types of bio-deterioration artefacts, such as tunnelling, vesicles, and fragmentation (Fig. 1d). The histological analysis of the hair cortex showed the presence of large oval-to-round-shaped structures known as ovoid bodies and pigment granules (Fig. 1e). In one of the hair shafts, it was possible to identify the medulla, a discontinuous line of cuboidal cells which run through the centre of the cortex. The medulla was observable under confocal laser scanning (Fig. 1e) and light microscopy (Fig. 1f). Characteristic was the lack of fungal attack on the medulla.

3.3. Soft tissues

Macroscopically, the soft tissues were brittle, extremely desiccated, and very thin (1–4 mm). Under scanning electron microscopy, remnants from the dorsal interosseus muscle were observed on the proximal epiphysis of the third left metacarpal (Fig. 2); despite the thinness of the samples, the muscle contained a large number of structures composed of several parallel layers. Histological analysis of the soft tissues also showed the presence of human erythrocytes, which were distributed in small aggregates and retained their classic biconcave form (Fig. 3).

3.4. Chemical analysis

The GC/MS analysis of the extracts of the second metacarpal, the hair sample, and the loose particles showed the presence of various substances, mainly sesquiterpenes, diterpenes and triterpenes, fatty acids, steroids, cinnamates, vanillin, and substances the exact origin of which was not identifiable (Table 1).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) and cinnamates were detected in the bone and the hair extract, whereas