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PHD SCHOOL IN BIOLOGY

INVESTIGATIONS OF ANCIENT HUMAN REMAINS USING COMPLEMENTARY METHODS IN MORPHOLOGY AND CHEMICAL ANTHROPOLOGY

SUMMARY OF THE PHD THESIS

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Introduction

The analysis of bioanthropological finds is a complex task: the biological reconstruction of a past population, the investigation of its lifestyle, health status and migration usually exceeds the scope of classical anthropological research (Martin and Saller 1957, Nemeskéri *et al.* 1960, Éry *et al.* 1963, Alekszejev and Debec 1964, Stloukal and Hanáková 1978, Finnegan and Marcsik 1979, Ortner and Putschar 1981, Isçan 1989, Sjøvold 1990, Aufderheide and Rodríguez-Martín 1998, Pálfi *et al.* 1999, Ortner 2003). The technical development of related scientific disciplines transformed anthropological research into an interdisciplinary field, providing new or improved tools for answering old questions. In the last five decades, state-of-the-art natural science and medical research techniques (including radiological, genetical, microbial and chemical methods) continuously claimed roles in the world of anthropological research (e.g. Lengyel and Nemeskéri 1963, 1964, Nemeskéri and Lengyel 1963, Lengyel 1975, Pääbo 1985, Kłys *et al.* 1999, Carvalho *et al.* 2000, Haas *et al.* 2000, Gearney *et al.* 2001, Degryse *et al.* 2004, Donoghue *et al.* 2005, Wilson 2005, Chan *et al.* 2013, Allentoft *et al.* 2015, Pálfi *et al.* 2015). Interdisciplinary cooperations facilitate complex anthropological investigations that provide useful complementary information to classical anthropological research concerning paleopathology, paleodiet, migration and bioarcheology, but occasionally also open completely new research perspectives.

The bioanthropological finds in the focus of our interest usually pose archeological questions that can only be answered using bioarcheological approaches or, in many instances, other related scientific fields need to be called in for help. Among these fields, we will pay special attention to chemical anthropology that lays in the intersection of chemistry and anthropology. It is also a new interdisciplinary field (Márk 2006, Boros-Major *et al.* 2011, Madgwick and Broderick 2016) that flourishes as a result of the joint efforts of specialists of different scientific fields. In a broader sense, chemical anthropology may encompass all those bioanthropological methodologies that involve instruments and techniques originally developed in chemistry, physics, geology, biotechnology, medicine or genetics, and the results are discussed within an anthropological context also using competences of other fields of science.

Aims

The aims of the research that yielded my doctoral thesis are as follows:

1. Investigation of the partially mummified remains of the neonate Nyárlőrinc-Hangár utca inv. no. 14426 using approaches of chemical anthropology.
2. Complex investigation of the Orosháza microregion, especially the Orosháza-Bónum, Faluhely site, using approaches of anthropology, paleopathology, paleostomatology, and also considering diet, immigration and dating with the help of complementary morphological, biostatistical, medical imaging techniques and analytical chemistry of trace elements.
3. Complementary morphological, medical imaging, paleomicrobial, paleohistological and trace element analysis of specific infections (tuberculosis, syphilis and leprosy).
4. Trace element analysis of archeological human hair samples from the Vác mummy series.

Material and methods

Our anthropological and paleopathological investigations targeted the remains of 698 individuals from 5 archeological sites, also including 4331 teeth of the same sample (Balázs *et al.* 2005, Rózsa and Balázs 2011, Rózsa *et al.* 2012, Rózsa *et al.* 2014a,b). The samples included the partially mummified remains of a neonate. We have also screened the data of 172 further skeletons (Lipták and Farkas 1962) to find more samples for comparative chemical analysis. When collecting hair samples, the whole Vác mummy series was assessed (Pap *et al.* 1997).

During the course of our investigations we have applied many macromorphological (e.g. Martin and Saller 1957, Finnegan and Marcsik 1979, Cockburn *et al.* 1998, Ortner 2003), paleoradiological (Chhem and Brothwell 2008), paleohistological (Schultz 2003), paleomicrobial (Zink *et al.* 2003, Donoghue *et al.* 2005) techniques, and several methodologies of analytical chemistry known to chemical anthropology (Kłys *et al.* 1999, Carvalho *et al.* 2000, Zlateva *et al.* 2003, Degryse *et al.* 2004, Wilson 2005, Giblin 2011).

Chemical analyses have been carried out on bone samples from 4 archeological excavations (Orosháza-Bónum, Faluhely, Orosháza-Rákóczitelep, Gádoros-Templomhely and Nagyszénás-Vaskapu), and mummified human remains from Nyárlőrinc and Vác. The investigations were carried out in the Department of Inorganic and Analytical Chemistry, the

Department of Mineralogy, Geochemistry and Petrology and the Department of Physical Geography and Geoinformatics of the Faculty of Science and Informatics at the University of Szeged. Some sample preparations and measurements were also carried out at the ATOMKI Institute for Nuclear Research, Hungarian Academy of Sciences (Debrecen), as well as in the Quinnipiac University (USA) and the Yale University (USA), in cases the special analytical infrastructure needed was not available at the University of Szeged. The bioanthropological remains derive from the collection of the Nagy Gyula Regional Museum in Orosháza, the Department of Biological Anthropology of the Faculty of Science and Informatics at the University of Szeged (Szeged Anthropological Collection, SAC), and the Mummy Collection of the Department of Anthropology of the Hungarian Natural History Museum (Budapest).

Results

1. I have morphologically described the partially mummified remains of the neonate from Nyárlőrinc-Hangár utca (inv. no. 14426) and performed detailed element analysis on these finds that are exceptional on the national as well as the international level. ICP-AES (inductively coupled plasma-atomic emission spectrometry) and the XRF (X-ray fluorescence spectroscopy) measurements on the green coloured remains proved that the mummification was caused by an elevated copper concentration originating from a corroded copper coin found in the ceramic pot together with the human remains. The copper concentrations values found both on the surface and also inside of the remains were more than 3 orders of magnitude higher than normal values. On the basis of the observed distribution of concentration I could also clearly reconstruct the placement of the copper coin at the time of entombment. I have pointed out several possible anaerobic processes that may have caused the corrosion of the copper coin under these special circumstances (Balázs *et al.* 2005, Balázs 2007, Balázs and Bölkei 2007, Balázs *et al.* 2016a,b,c).
2. I have performed general anthropological and paleopathological characterization of 4 human skeletal series from the Orosháza microregion (Rózsa and Balázs 2011, Rózsa *et al.* 2012, Rózsa *et al.* 2014a,b, Balázs *et al.* 2015b). Using biological distance calculations, I have proved that the series from Orosháza-Bónum, Faluhely is profoundly different from the rest of the regional osteoarcheological material in the same era (Balázs and Lovász 2016).
3. Using ICP-MS (inductively coupled plasma mass spectrometry) trace element analysis,

I have found evidence that even archeological bone materials affected by taphonomical processes exhibit Ca/P concentration ratios that increase in correlation with the age at death, similarly to modern bone tissue. I have pointed out that such analytical data considered within one particular population excavated from the same soil may provide a basis for the estimation of age groups at death (Balázs *et al.* 2015c).

4. ICP-MS trace element analysis proved that the past population from Orosháza-Bónum, Faluhely had more meat and animal protein in their diet than the average of the microregion of the respective era. We can also track down how the share of vegetables in the diet continuously increased within a century (Balázs *et al.* 2015c). Ongoing ICP-MS-based ^{87}Sr and ^{86}Sr isotope measurements aim at finding the first generation of settlers of this population. Our preliminary results have already started to outline averages characteristic to the Orosháza microregion and some individual differences.
5. Liquid scintillation counting of carbon isotopes did not provide sufficient results to accurately date the Orosháza-Bónum, Faluhely site that has formerly been dated to the Árpádian Age (11-13th century AD) on the basis archeological features. Neither conventional, nor calibrated dates were obtainable. Trace element analysis cannot provide chronological dating, but it can be used to explore taphonomical effects. Based on taphonomical data, I have come to the conclusion that the cemetery of Orosháza-Bónum, Faluhely can be separated into 3 waves of inhumations, and entombments were initiated in the southern part continuing northwards (Balázs *et al.* 2015c).
6. Among specific infectious diseases, tuberculosis and syphilis can be observed in the Nyárlőrinc-Hangár utca series (Balázs *et al.* 2005, Balázs *et al.* 2015d), and the osteological symptoms of leprosy has been detected in the Orosháza-Bónum, Faluhely series (Balázs *et al.* 2015c). The morphological diagnosis has been proven applying medical imaging techniques, paleomicrobial and paleohistological methods. The remains of the leprosy patient have been subjected to chemical analysis, thus the presence of the disease in the 13th century has also been confirmed (Balázs *et al.* 2015c).
7. I have investigated hair samples from the 18-19th century mummified remains found in the crypt of the Dominican Church (Fehérek temploma) of Vác, Hungary. Measurements of trace element contents in the hair samples of this collection using a LA-ICP-MS (laser ablation inductively coupled plasma mass spectrometry) are the first in the literature. I have examined samples from 6 mummies, and determined the average concentrations, as well as longitudinal and cross-sectional distributions of a

total of 9 trace elements (Cu, Zn, Sr, Hg, Pb, Al, Fe, As, Ag). I have extensively discussed possible explanations of the observed distributions, and their tentative sexual, age-related, and occupational differences. I have concluded, that the average concentration of Pb and Al in the mummy samples was considerably higher than in modern samples, but none of the trace elements showed such high concentrations that may indicate poisoning. Cross-sectional distribution of trace elements showed that only Cu was accumulated in the surfacial layers of hair. It has occurred in several instances that co-accumulation of certain trace elements was detected along the hair or in the cross-section (Balázs *et al.* 2017).

Discussion of the results and perspectives

Using μXRF and ICP-AES techniques, I have confirmed that copper played a definitive role in the partial mummification observed on the remains of Nyárlőrinc-Hangár utca inv. no. 14426 neonate. By doing so, we have described a new type of mummification process. It has been unambiguously proved that the quasi-natural mummificiation process was initiated by copper dissolved from a 19th century coin placed in the palm the deceased, which must have been a characteristic grave good in burials of unbaptized infants in the era (Dömötör 1990, Selmeczi 1992). Copper inside the pot that contained the remains of the infant may have locally blocked decomposition.

I have also published a lot of new data concerning metrical and non-metrical characteristics, paleostomatology, and paleopathology of the biarcheological remains of the Orosháza microregion. The Orosháza-Bónum, Faluhely excavation site is the first (and currently the only) Árpádian Age Muslim burial complex studied in Hungary, hence, all published data may be considered unique. The paleopathological observations have yielded new data primarily concerning interdisciplinary investigations of specific infectious diseases (leprosy, syphilis and tuberculosis).

During the course of our chemical examinations I have successfully adapted cleaning, digestion and measuring methods using ICP-MS technique to measure the trace element composition of archeological bone samples. My results have proved that the Ca/P concentration ratio increased with the age at death of the individual, and this phenomenon is not only present in modern samples, but in archeological context too. In bigger quantities, this type of measurement within one population may provide a basis for the estimation of age groups at death. Analytical measurements of Ba, Sr and Zn trace elements provided

information about the diet of this skeletal population. On the basis of trace element measurements, I have defined a relative chronology of inhumations in the Orosháza-Bónum, Faluhely cemetery, where direct archeological dating of a lot of graves containing no grave goods was not possible. The carbon dating of the cemetery is under way. The preliminary measurements of $^{87}Sr/^{86}Sr$ isotopic ratio in the bioacheological remains of Orosháza-Bónum, Faluhely show that at least parts of this population may have originated from a different geographical environment than where they were buried.

I have pioneered in the practice of Hungarian mummy studies using LA-ICP-MS measurement technique to determine trace element contents of hair samples. In a preliminary study, I have measured hair samples from the Vác mummy series, and analyzed the data from the viewpoint of surviving written sources and interdisciplinary mummy research too.

Some parts of this research are still ongoing, and we are also planning on including more samples in certain measurement procedures. In order to obtain a more detailed picture, the liquid scintillation carbon dating will be confirmed by a new set of samples and the inclusion of a parallel laboratory. In case of the graves without grave goods in the Orosháza-Bónum, Faluhely cemetery, it would be very important to narrow down the carbon dating to shorter and more accurate periods to see their relationship with the demolition of the village at the time of the Mongol invasion. The trace element results, however, fit the archeological phenomena found in the village very well.

Mine $^{87}Sr/^{86}Sr$ and ^{14}C isotopic measurements provided an opportunity to join the international trend of exploiting this revolutionary indicator useful for population migration research and bioanthropological dating. In recent years, very few trace element studies have been published concerning Hungarian archeological bone samples. My studies demonstrate how investigations in chemical anthropology are not only complementary to other fields of bioarcheological research, but in many instances they are invaluable in the evaluation of a finding or a burial.

As a new research direction, we are planning on ICP-MS trace element measurements in tooth enamel samples at the Department of Inorganic and Analytical Chemistry of the University of Szeged, and C, N, and O stable isotope analyses at the Quinnipiac University (USA), in order to draw a clearer picture on the diet of Muslim communities that lived in the Árpádian Age.

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Coauthor's/Director's declaration

I, undersigned Dr. György Pálfi, PhD, head of the Department of Biological Anthropology, University of Szeged, first author of the mentioned publication and director of Mr János BALÁZS' PhD work hereby certify that I am familiar with the PhD thesis of the applicant Mr János BALÁZS (University of Szeged) entitled **'Investigations of ancient human remains using complementary methods in morphology and chemical anthropology'** *("Komplementer morfológiai és kémiai antropológiai vizsgálatok régi emberi maradványokon").*

Regarding our jointly obtained and published results that form part of this PhD dissertation, I declare the followings:

The applicant's contribution was prominent in obtaining the following results:

Morphological study and interpretation of the biochemical data from the biomolecular study of a 7-8th century spine (Pott's disease, Collection of the Department of Biological Anthropology, University of Szeged).

I did not and will not use these results in getting an academic research degree. There is no other PhD student who can use these results in a doctoral process.

Regarding our joint results referred to in this thesis, the following one was obtained as the result of joint contribution by the applicant and myself:

Pálfi György, Maixner Frank, Maczel Márta, Molnár Erika, Pósa Annamária, Kristóf Lilla Alida, Marcsik Antónia, **Balázs János**, Masson Muriel, Paja László, Palkó András, Szentgyörgyi Réka, Nerlich Andreas, Zink Albert, Dutour Olivier: Unusual spinal tuberculosis in an Avar Age skeleton (Csongrád-Felgyő, Ürmös tanya, Hungary): a morphological and biomolecular study. *Tuberculosis* 95 (2015). S29–S34. p.

I attest that the above statement is true and valid to the best of my knowledge.

Szeged, February 05th 2017.

(Dr. György Pálfi, PhD, Head of Department)