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EXPERT REPORT

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Mission Reminder

As part of *The Alien Project*, five samples of different mummies parts body found site of Nazca (Peru) were sent to me for examination of their molecular composition by mid-infrared spectroscopy. This technology makes it possible to identify the (bio) chemical composition of any sample made of organic matter. The present expertise aims to analyze these mummified fragments.

The analysis were conducted at the Dupuy de Lôme Research Institute (UMR CNRS 6027) in Vannes (France).

Samples were listed as follows:

- 01 VICTORIA'S BONE (hip) 02 – HAND TENDON (tHREE FINGERED HAND) 03 – HAND BONE 04 – MARIA'S SACRUM
- 04 = MARIA'S SAC05 = MARIA'S HIP

Expertise Methodology

The five samples were sent in small plastic tubes or flasks. A first step was to make high magnification pictures. The shots below were obtained with a Nikon D610 camera and a NIKKOR105 1: 2.8 MACRO lens. To indicate the scale, an 8 mm diameter coin cell was systematically photographed with the objects. High-resolution shots are available as a separate compressed file from this report.



Fig.1 VICTORIA's BONE (two faces)



Fig. 2 HAND TENDON (two sides)



Fig. 3 HAND THREE FINGERED BONE (two sides)



Fig. 4 MARIA'S SACRUM (two faces)



Fig. 5 MARIA's HIP (two faces)

Infrared spectroscopic analysis

The samples were then analyzed in mid-infrared spectroscopy (MIR) using a Lumos (Bruker) infrared microscope in micro ATR mode.



FIG. 6 LUMOS Micropectrophotometer

Typically, this absorption spectroscopy makes it possible to identify the chemical groups present in the sample of interest through their vibrational modes. The chemical groups (O-H, C-C, N-H, C = O, etc.) have different absorption bands in their position in the spectre en fonction of



the mass of atoms being connected by a covalent chemical bond and the type of bond (single or double). The figure above shows the main spectral domains reflecting the respective contributions of proteins, lipids (fats), sugars or nucleic acids (DNA, RNA).

For comparison purposes, different "reference" samples were analyzed. These include samples of muscle, skin and chicken bones placed in a desiccator for two months and a moult of viper (Daboia palestinae). The corresponding snapshots are shown below.



Fig. 7 Viper moult (D. palestinæ) Fig. 8 Dehydrated chicken muscle



Fig. 9 Chicken Bones (Two sides)



Fig. 10 Dehydrated chicken skin (two sides)

Acquisitions Infrarouge

The different samples were analyzed by single-reflection ATR by MIR microspectroscopy using a Germanium crystal. The samples are brought into contact with the crystal; the sampled area is about 2000 μ m2 over a 2-3 μ m thickness; It is therefore essentially a surface analysis. For each analysis, 128 spectra are averaged with a spectral resolution of 4 cm⁻¹.



Pretreatments of the spectra. In order to optimize the spectral resolution, the second derivatives of the spectra are calculated and a vector normalization is performed. This makes it possible to compensate absorbance variations only due to the material "quantity" seen by the crystal. These second derivatives are used for multivariate statistical analysis.

During the acquisitions, some of the small boiling spots that characterize evaporation of water have been noted. This phenomenon is due to the fact that the light of the microscope (lit before the MIR acquisition itself) is concentrated on a very small surface which causes a local heating. It can be deduced that there are small pockets of water trapped probably in a hydrophobic lipidic environment in the samples, therefore waterproof. Fusion of the lipids under the heat of the light would make the evaporation of the water possible.



Fig. 11 Spectrum MIR type (here a bacteria) with its second derivative (in blue)

Spectral analysis

The individual spectra are first analyzed to better identify their biochemical components (proteins, fats, sugars, ...). To account for spatial heterogeneities (at mm scale), several acquisitions (3 to 5) were performed on each sample. The most representative spectra are presented. For all samples, both sides were analyzed..

Fig. 12 VICTORIA's bone



This sample has a very irregular face (left Fig.1, black and red spectra) and a clearly alveolated face (right Fig.1, green spectrum).

The irregular face is heterogeneous with the presence of proteins (amides I and II) at 1540 and 1650 cm⁻¹, mineralized tissue (carbonate band CaCO³⁻ at 860-870 cm⁻¹) and esterified lipids (1740, 1420, 1320 and massive 2800-3000 cm⁻¹). The honeycomb face is dominated by lipids with a very important ester band and an intense 1240 band. The 1010 band should reflect the phosphate group that is characteristic of bone tissue. The latter must be linked to the alveolar structure clearly visible in Fig.1 (right).





The "Hand tendon" spectra (Fig.2) collected on each of the faces (blue-green-yellow vs blackred) are qualitatively close except a stronger contribution of the phosphocalcic matrix. (Mass at 1030 cm⁻¹ and band at 800 cm⁻¹) Lipids are detectable at 1740 and 2800-3000 cm⁻¹. The 1230 cm⁻¹ band cannot be uniquely assigned.

The lack of signature of the characteristic tendon proteins (collagen and elastin) suggests that these constituents have been destroyed or that the sample does not specifically correspond to this tissue but rather to bone tissue.





This sample (FIG. 3) has a relatively unified and smooth appearance on one of these faces for two fragments, whereas the opposite face has a fibrous appearance. We will consider that the smooth side corresponds to the external surface of the bone, the fibrous aspect to the internal face.

The black and red spectra correspond to the external face. These spectra are dominated by a broad and intense band (1030 cm⁻¹) representing the vibration of the phosphate groups (PO_4^{3-}) characteristic of the phosphocalcic mineral matrix of the bone. The shoulder and small peak around 880 cm⁻¹ reflect carbonates. The 1640 cm⁻¹ band is representative of collagen, which is the most abundant protein in bone. The band at 1320 cm⁻¹ (visible on the red spectrum) and the bands between 2800 and 3000 cm⁻¹ reflect lipids (fats).

Spectra acquired on the inner side of the bone are in turn largely dominated by lipids, which are the major constituents of the bone marrow. Presence of triglycerides in high concentrations is signed by intense bands between 2800 and 3000 cm⁻¹ (methylene groups CH₂ and methyl CH₃ fatty acids) and at 1740 cm⁻¹ (bond C = O ester glycerol-fatty acid triglycerides). There is also an intense band at 1240 cm⁻¹ which signals the asymmetric vibration of CH₂ in phospholipids.

In contrast to the outer surface, the proteins are virtually absent while the phosphocalcic band (1030 cm⁻¹) is twice less intense.



The MIR spectrum of this sample (Fig.4) is dominated by the massive at 1030 cm⁻¹ which shows the bone tissue. No trace of lipids or proteins is detected. It is worth mentioning a doublet at 660-670 cm⁻¹ which is generally attributed to the sulfur group S=O. Presence of sulfur is not recorded in the mineral part of the bone. This attribution may therefore be subject to caution.

Fig. 16 MARIA'S Hanche



This sample has two faces of different aspects. The face shown in FIG. 5 (right) is mainly characterized by a phosphocalcic band at 1030 cm⁻¹ (black and red spectra) with a visible lipid contribution between 2800 and 3000 cm⁻¹. The other side (yellow, cyan, magenta and blue spectra) is essentially lipidic nature (2800-3000 and 1710 cm⁻¹) with a presence of proteins visible by amide I (1640 cm⁻¹) and II (1540 cm⁻¹) characteristics of these macromolecules.

We thus find the characteristics of the bone tissue with one side essentially mineral and the other marked by the lipids of the bone marrow.

Comparative analysis

In order to have reference tissues as close as possible to the samples, different tissues (chicken and snake moult) were analyzed. The chicken tissues were previously placed in a vacuum desiccator for two months in the presence of silica gel. The snake moult was analyzed as its own. The different spectra obtained are presented below.



Fig. 17







The highlight of these comparisons between mummy samples and reference samples is the near absence (or low concentration) of the proteins found in the mummy samples. This fact could be explained by a chemical degradation of the proteins which would result in a lysis of the peptide bonds which connect the amino acids to each other (a protein is a polymer of amino acids). Indeed, as mentioned above, the peptide bond absorbs in MIR at 1640 and 1540 cm⁻¹. These "Proteins" bands are only visible in samples from Maria and Victoria's hip.

It will also be noted that the internal faces of the analyzed bones have a honeycomb structure.

In order to highlight the similarities and di-similarities between samples, a principal component analysis (PCA) was performed. This analysis, by making it possible to reduce the dimensionality of the MIR spectra, makes it possible to calculate an "Euclidean distance" between two spectra represented in a space (here) with two dimensions. The distance between two bipoints reflects the greater or lesser proximity between two samples. The figures below, called factorial maps, show the distribution of individuals (spectra).

Samples codes:

VH E/I	Victoria's Bone (hip) External face/ Internal
MT	Hand Tendon
MO E/I	Hand Bone Facial External / Internal
MS	Maria's Sacrum
MH E/I	Maria's hip External face / Internal





Figure 21 (Left) shows the factorial map of all samples from the mummies. It is very clear that the MOI (Hand Bone internal face) samples are very different from all the others. These differences are evident when comparing their negative (x-coordinate) scores (about -0.3) to the zero-scores of the other samples. The percentage displayed on each axis represents the % variability represented by a given main component; 54% for PC1 and 16% for PC2 in this case.

A second PCR was therefore performed to "dilate" the other samples; this is what Figure 21 right represents. The MT samples form a distinct cloud (or cluster), as well as the MHI and MHE samples. The MOE, VHE / VHI and MS samples are close to each other.

A first observation is that Maria's and Victoria's hip samples are distinct. Secondly the outer side of the tridactyl Hand Bone (MOE) is close to HEV, a little less than MS..

To locate these samples against a set of "references" the dehydrated samples of chicken and snake moult were projected on the factorial map constructed from the only samples from the mummies.

For references, the codes are:

Chicken Bone Face
Chicken Skin
Chicken muscle
Scale moult of viper
Tegument moult of viper
Internal face of the viper moult



Fig. 22

These "references" are here projected in red. We see that they have very low scores along the PC2 (y) axis and that this axis only shows 1% of the variability of these references. This means that these samples are poorly described by the PC2 model. This is not the case with PC1 although the representativeness of CP1 remains low (13%).

Although these "reconciliations" are unreliable, we note the proximities of the MOE (Hand External Bone) sample with viper moult samples.

General conclusions

- 1. The spectral analysis carried out in the context of the present report demonstrate beyond any doubt that the samples submitted correspond to highly dehydrated biological samples.
- 2. Only the nature of the tissue corresponding to Hand Tendon seems to correspond more to bone tissue than to a tendon, this type of tissue being essentially composed of proteins, collagen for the most part. This may be similar to bone tissue which, as noted, has low or non-detectable levels of protein.
- 3. Attempts to reconcile with dehydrated samples in the laboratory are inconclusive. It would be interesting to be able to compare the viper moult spectra with a skin sample of the reptilian species.
- 4. The Hand Bone (MOE) sample seems to be closer to Victoria's (HEV) than Maria's; this point is to be checked because the expert is not aware that the Hand Bone sample has been identified as belonging to a particular species.
- 5. If more precise assignments are needed, they should be based on a larger number of samples with a precise knowledge of the type of tissue. This applies to the samples of the mummies because, as we have seen, the comparison with dehydrated samples in the laboratory is inconclusive.
- 6. The point about proteins, very little present, would also be to invest on proven samples like muscle.

Fait à Vannes, le 30 juillet 2019 Olivier SIRE Expert