Observations on the diagenesis of dwarf elephant skeletal remains from the island of Tilos (Dodekanese, Greece)

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SUMMARY: The object of this analysis is the study of the diagenetic alterations that appear in bones from the Charkadio Cave on the island of Tilos (Dodekanese, Greece) that have been provisionally attributed to *Palaeoloxodon antiquus falconeri* Busk. The bone samples were studied through optical and scanning electron microscopy, X-ray microanalysis and X-ray diffraction, while the Ca/P ratio and Crystallinity Index (C.I.) were estimated.

1. Introduction

Bone is a heterogeneous, complicated dynamic system which consists of a dense framework of organised fibrils of collagen and a poorly crystallised hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$) which always contains a certain amount of carbonate and thus is called carbonate hydroxylapatite (Posner 1985; Person *et al.* 1995). This biological apatite is characterised by a greater ion exchange than that of the mineral apatite, due to its smaller crystals. This leads to a significantly different chemical composition and the presence of many elements (Weiner & Price 1986). Bones crystallinity seems to increase with age, during both the animal's life and its journey from the biosphere to the lithosphere (Bonar *et al.* 1983). The procedures that lead to the preservation of bone through time are known as diagenesis and have been the object of many studies during the last decades (Hill 1980; Clarke *et al.* 1993; Hedges *et al.* 1995a, b). Diagenesis can be recognized by a number of alterations that occur at different stages from the time of death and which depend greatly on the existing geochemical conditions (Marean 1991).

These alterations may concern changes of bone histology and its preservation, the amount of organic matter within, its size and shape as well as the presence of intrusions and void filling by mineral phases. These last two changes are often connected to microbial activity, which occur from the very first hours postmortem. Dissolution and recrystallization of the apatitic phase and numerous ionic exchanges with the environment are also possible alterations, which in turn may lead to a differentiation of the Ca/P ratios, the crystallinity of bone etc (Hackett 1981; Piepenbrink 1989; Grupe 1995; Person *et al.* 1995).

2. Materials and Methods

The material used in this study belongs to dwarf elephants that were originally attributed to the subspecies *Palaeoloxodon antiquus falconeri* Busk by Symeonidis (1972). Theodorou (1983) discusses the problem of using the same taxon for Tilos and Malta elephants and accepts it temporarily. Material coming from the last excavations will allow us the detailed description of the elephants and possibly their correct attribution to another taxon. These dwarf elephants dominate the cave fauna and which make it the richest dwarf elephant site in the world. Only 20% of the sediment coming from the first of the two known chambers has given an estimate of more than 40 elephants. These elephants are also considered to be the last elephants in Europe, as their appearance in the cave ranges from 45 to 4-3.5 ka. The rest of the cave fauna consists of deer (140 ka old), chelonia, aves and some micromammals (Symeonidis 1972; Bachmayer *et al.* 1976; Theodorou 1983, 1988).

Six bone samples where chosen (Fig. 1) from which samples 1-4 are costae and samples 5-6 are metapodials. Samples 1, 2 and 3 were taken from the region close to the cave wall (E position; Symeonidis 1976) and were covered by hardened cave deposits. The depth from which they were derived is 1.6 m from zero point for the first two and 0.7-1.1 m for sample 3. Sample 4 was dug up from square Q9 and the depth of 2.9 m from zero point and appears to be darker on the outer side than the rest of the samples. Samples 5 and 6 come from the depths of 0.7-1.3 m and 1.4-1.8 m respectively.

Thin sections of the above samples were studied both by optical and scanning electron microscopy, so as to observe the state of preservation of the internal structure of the bone and the mode in which diagenesis has affected it. For the same purpose, pieces of compact bone of each sample were also observed using scanning electron microscopy, after being covered by gold. Chemical analysis of both the samples and their surrounding sediment were obtained by X-ray microanalysis (EDS), while X-ray diffraction provided us with their mineralogical composition. The crystallinity index for each bone was also determined by means of this technique according to the method proposed by Person *et al.* (1995).

3. Results and Conclusions

The observation of our material under the microscope leads us to believe that diagenesis has not affected bone structure significantly. All regions of compact bone are covered by healthy osteons, which appear with all their characteristic features (Figs. 2, 3). There are no signs of microbial activity, while there are few signs of stress, which have lead to some cracking and deformation (Fig. 4). Apart from the apatitic phase one may observe voids (haversian tunnels, lacunae et canaliculi, cracks etc.) filled mainly by calcite (Figs. 2, 5) while small quantities of the surrounding sediment seem to have entered the structure in some regions.

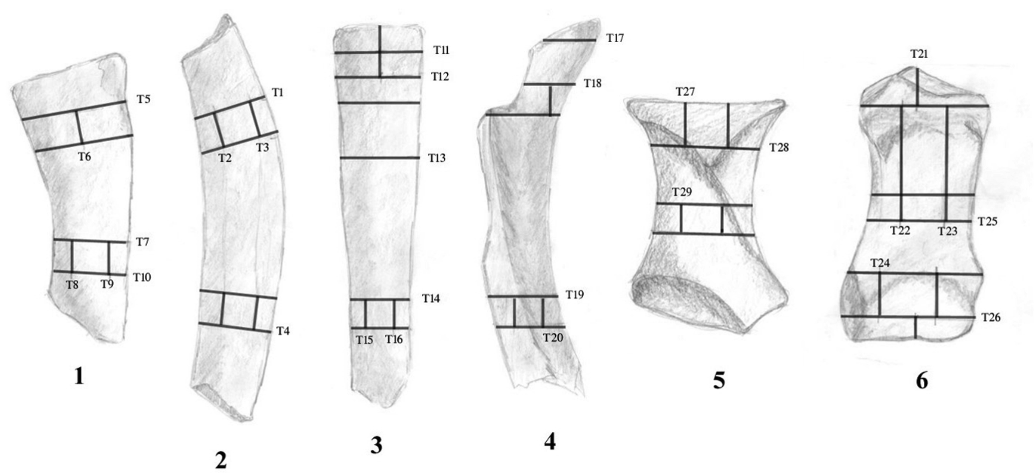

Fig.1 - The material selected for this study (33% of actual size). One may observe the position of the thin sections made on each bone.

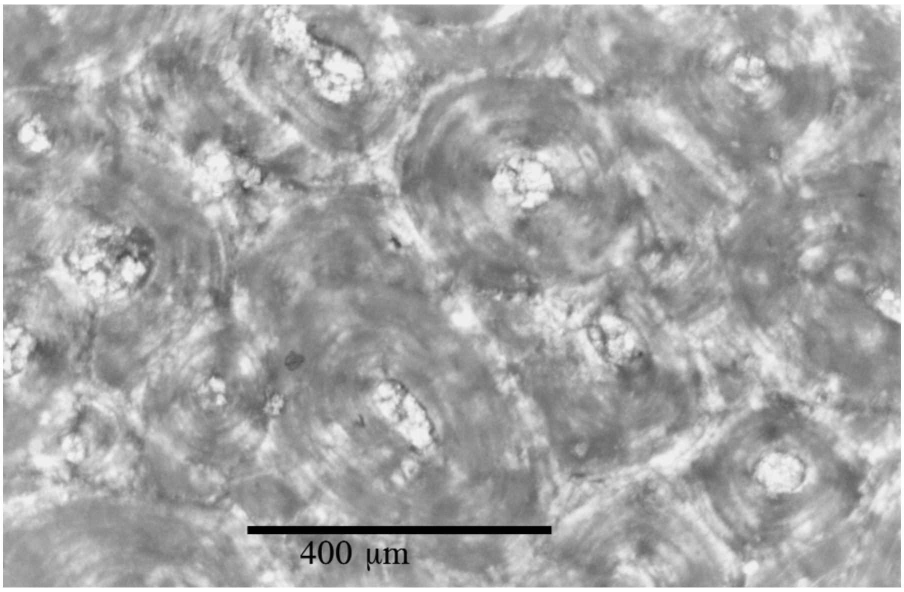

Fig.2 - Haversian systems under polarized light (section T4). Calcite filling all voids might also be observed.

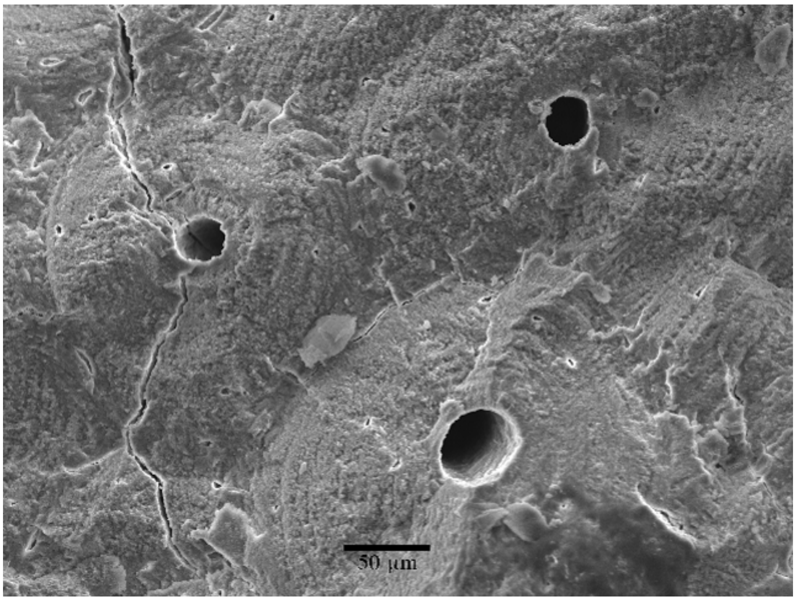

Fig.3 - The same features seen through the SEM (sample 4).

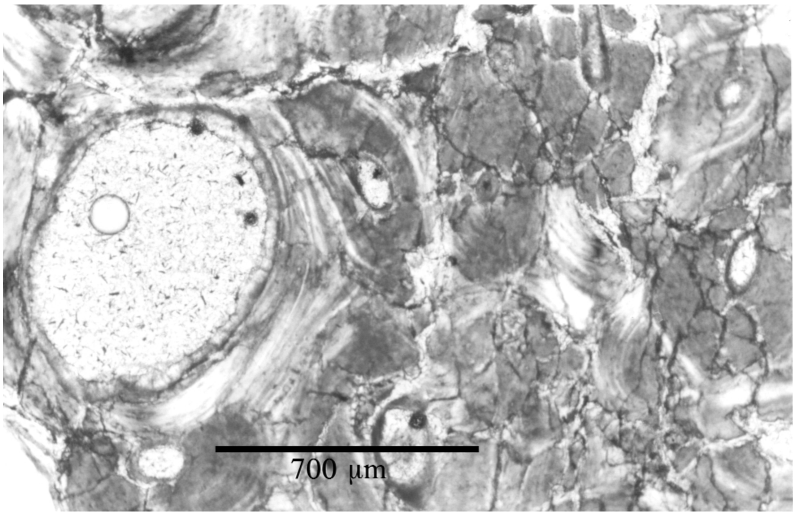

Fig.4 - Osteons of different sizes near the cancelous bone region under polarized light, with obvious signs of cracking (section T28).

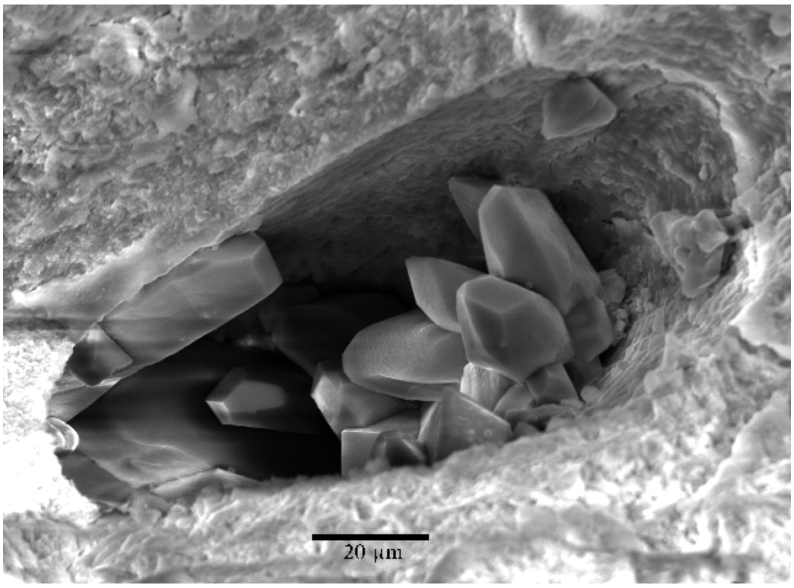

Fig.5 - Calcite crystals inside a haversial channel, seen through the SEM (sample 1).

The use of X-ray diffraction also proves the presence of other mineral phases apart from hydroxylapatite, which does not contain any fluorine. Calcite seems to dominate, since it appears in 5 out of 6 samples. Quartz is also present in smaller quantities (Tab. 1, Tuross *et al.* 1989). This conclusion derives from the value of the ratios given in Table 1. Their values are given by the division of the heighest peak of the mineral phase of interest by the highest peak of hydroxylapatite. The crystallinity index value is given by the sum of the heights of the peaks corresponding to reflections (211), (112), (300) divided by the height of the highest peak (211). It ranges between 0.2 and 0.44, and proves that the recrystallization of the biological apatite has already begun. These indexes are quite close to that of modern bone (0.0), a fact that generally characterizes material coming from cave deposits.

Tab.1 - Crystallinity indexes for each sample, as well as the ratios of calcite/hydroxylapatite and quartz/hydroxylapatite estimated by the XRD technique according to Tuross *et al*. (1989).

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Average
Crystallinity Index	0.3	0.2	0.26	0.44	0.22	0.27	0.28
Calcite/Hydroxylapatite	0.37	1.22	1.02		1.08	0.81	0.75
Quartz/Hydroxylapatite		0.15		0.16		0.31	0.1

Tab.2 - Average Chemical analysis (%) for each sample. All measurements of elements are in oxides except for Cl.

Oxides	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
SiO_2	0.81	1.87	0.25	1.26	0.93	0.46
Al_2O_3	0.23	0.22	0.12	0.26	0.22	0.23
MgO		0.44		0.47	0.32	
CaO	57.95	55.88	60.62	54.75	55.97	57.47
Na_2O	0.54	0.8	0.48	0.63	1.06	1
P_2O_5	38.02	39.26	36.97	41.49	38.86	38.33
SO_3	0.6	0.93	0.53	0.76	1.85	1.59
Cl	0.47			0.16	0.17	0.11
TOTAL	98.62	99.4	98.97	99.78	99.38	99.19
Ca/P	2.53	2.34	2.71	2.16	2.38	2.48

The surrounding sediments consist mainly of calcite, quartz, feldspars and clay minerals such as illite, montmorillonite etc. Their chemical composition shows apart from the above, quantities of phosphorous that range between 1.98% and 29.03%. This proves that bone has lost some of its P during its interaction with its surrounding media while this was obvious also by the Ca/P ratios.

Because of the mentioned intrusions and the interaction of the bone hydroxylapatite with the surrounding environment, the chemical composition of our samples shows an increase in elements such as Al, Si, Ca while others (Na, Mg, K) already exist in bone due to physiological needs ante mortem (Tab. 2). Ca/P ratios are quite close to those of modern bone (2.16) and are often increased due to the presence of calcite or the depletion or substitution of phosphorous (Stathopoulou 2000, and references within). Although the cave contains important layers of volcanic material, there seems to be no affect on the composition of the skeletal remains with Si appearing in relatively small quantities.

From the above, we conclude that the processes of diagenesis have obviously begun to affect the material being studied but are still at a primary stage. It is also obvious that some of these processes have affected the bone more than others. Further study will give us a clearer picture of the conditions that lead to the observed state of preservation and alterations.

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