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Skeletal Remains Submerged in Mediterranean Sea for Eight Years: Histological Observations

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Abstract

The authors describe the histological changes observed in a number of bone fragments which had remained submerged at a depth of 3850 metres in the Mediterranean Sea for 7 years and 10 months. The bone fragments which presented a complete skeletization were without any cellular residue while collagen fibres were still appreciable above all in the outermost circumferential lamellas of each osteonic system. No presence of extraneous biological material such as fungi, bacteria or other micro-organisms which often colonise buried bones were found on any of the bone structures examined. The bone remains moreover exhibited a notably reduced density due to the notable loss of inorganic constituents.

Keywords: Collagen fibres; Histological modifications; Submerged bones

Introduction

The post mortem histological modifications of human bones and the time in which they are carried out have been widely described and reported in literature [1-6] but such remarks refer to buried bone remains, exposed to the action of various climactic factors and/or the micro-macro fauna [7,8], but few studies concern the modifications of bones immersed in water.

Besides, many techniques for the extraction of DNA from bones have been developed [9-11] and Krainic [12] described a DNA extraction from skeletal remains submerged in water for three years [13]. Nevertheless we have not been able to find in any of the literature descriptions of the histological changes that occur in human bones submerged in seawater over a notable period of time.

For this reason it seemed useful to describe in this paper the histological modifications of human bones submerged in the Mediterranean Sea for around 8 years.

Material and Methods

As part of the recovery operation on a DC9 aircraft which had crashed into the Mediterranean 7 years and 10 months before, a number of bone fragments which could be traced to a diaphysis and/or an epiphysis of long bones were recovered among the metallic parts of the aircraft at a depth of 3850 m. The bone fragments were completely free from any tissular residue, had a white-greyish color and the typical bone consistency was well-maintained. The medullary canal was free from any adipocere residue.

Radiological Investigations

The fragment from a long bone epiphysis underwent a computerised tomographic examination which displayed the good state of conservation of the bone structure. The bone density expressed in HOUNSFIELD units was 326 H.U., where the normal levels for epiphysis go between 700 and 800 H.U.

Histological Investigation

After having been preserved in formol with Lillie's tamponade (40% formaldehyde: 100 ml; H_2O: 900 ml; monobasic sodium phosphate, monohydrate: 4 g; anhydrate bibasic phosphate: 6. g) for a few days, the fragments were decalcified in formic acid with Evans and Krajian tamponade, following Bancroft and Stevans' directions (20% water solution of trisode citrate: 65 ml; formic acid 90%: 35 ml). Tissues were routinely processed for paraffin embedding.Sections of 7 µm were stained with hematoxylin/eosin. The distribution of the collagen bundles was microscopically evaluated after Picrosirius staining by polarisation microscopy [14].

Picrosirus solution (Sirius red F3BA 1mg/ml in picric acid aqueous saturated solution) specifically enhances the collagen birefringence.

We have evaluated the fibrous collagen framework and identified the tissue patterns of distribution of collagen fibre according the following classification:

A type area is characterised by an absence of collagen fibres not exhibiting a particular interference colour by polarization microscopy after Picrosirius staining. B type area contains a loose presence of collagen fibres with greenish interference colour by polarisation microscopy after Picrosirius staining. C type area exhibits collagen bundles with a bright red interference colour by polarisation microscopy.

Results

The morphological changes noted featured the presence of numerous canalicoli in the outermost layers of the ossuary compacta which gave the surface of the bone a "grain-like" aspect (Figure 1).

In the underlying layers, on the other hand, it was possible to note the presence of the classic lamellar structure and some osteonic cavities.

The surfaces of the outermost lamellar seemed rough and uneven

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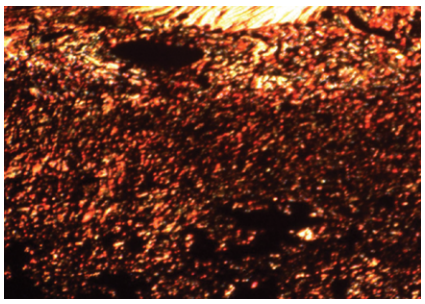

Figure 1: Surface of the Bone.

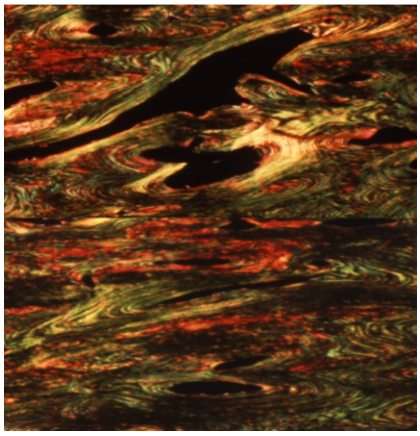

Figure 2: Collagen Fibres in Osteonic system.

due to minute microcavities. The more internal ones on the other hand showed a regular surface.

The internal layers of the bone surface instead showed clearly evident osteonic systems with the relative lamellar disposition.

All the osteonic cavities observed appeared optically empty, free from any cellular residue.

The presence of collagen fibres was however unevenly distributed, being clearly notable and more marked in the observations made of the subendostal surfaces (C Type Area) and less evident in the observations made of the subperiostial surfaces (A/B Type Area).

Moreover, the collagen fibres were present in the larger number of the outermost circumferential lamellar of each osteonic system, while the lamellar in closet contacts with the osteonic cavity were either scarcely present or completely absent (Figure 2).

DNA Investigation

The quantity of DNA extracted from degraded skeletal remains using the method described by Loreille [9] by Lee [10] by Fabbri [11] and by Krainic [12] was low and very degrated. It was impossible for us to restore a DNA profile.

Considerations

The first consideration to be made regards the total absence of any tissular fragment (cartilaginous, tendinous or muscular) from any of the bone remains observed, thus leading us to suppose a state of complete and total skeletization.

Actually, the processes of skeletization are highly variable and can occur even over an extremely brief span of time [13,15] and in particularly cold places [16], due to numerous factors relating to the body itself or the environment in which it is preserved.

The data that can be inferred from the literature [17] usually fix, in order to block any such phenomena, a maximum limit of 5 years for buried bodies and of 10-15 years for bodies in metallic casks.

Recent observations have however revealed how the processes of skeletization, when the corpse is positioned under earth, for a span of time such as that in consideration (7 years and 10 months), are never as pronounced as those observed, frequently being a state which could be defined as pre-skeletization or of incomplete skeletization.

In a series of 79 exhumations carried out on bodies that had been buried from 7 to 9 years, Canepa and his colleagues [18] have noted a percentage of skeletization of 79.8%.

In particular, in the case of bodies buried from between 7 and 8 years, the percentage was 72.4% and this was correlated to the state of permeability of the ground in which the body had been buried. The depth of the burial seemed also to be a significant factor in favouring the process of skeletisation [19].

In this case, even taking into account the meagreness of the bone fragments observed, it would certainly be possible to affirm that a corpse in seawater for 7 years and 10 months would be, even taking into consideration the action of the specific fauna, destructive enough to condition complete skeletization.

The structural alterations noted however displayed, as is typically observed in buried bones, a more pronounced destructive process on the periosteal surface where numerous micro-cavities had been present which tended to flow together and invade the underlying bone structure. That is to say, if the destructive process of the organic structures on the buried bones is favoured by the intervention of numerous micro-organisms (mycetes, bacteria, etc.) which actively participate in the destruction of the bone and which often can be traced in the context of the bone structure in the form of microaggregates [20], in the current case we found no presence of extraneous material coming from the external environment such as fungi, bacteria and other micro-organisms which generally colonise buried bones.

In our opinion, the unfavourable temperature of the water and the notable depth to which the bone remains were exposed for so many years make such observations sufficiently reasonable.

Another thing to be noted is the total absence in the bone structures of any cellular element or residue.

This is the consequence of a phenomenon, as Pierucci [21] has noted, that is essentially autolithic and which, taking place in the context of bone cavities, is usually hardly influenced by the external environment. That is to say, in the case in question, the permanence of the bone remains examined in seawater for a considerable period of time (7 years and 10 months) at a depth of 3850 metres, assured that they were exposed to the continual action of water which could permeate the bones as far as their deepest cavities.

Such a result, through various factors, such as the differing osmotic pressure of seawater, the different pH, the same mechanical erosion, and last but not least the diluting effect of the water have acted negatively on the cellular structures favouring their rapid decay and the consequent distancing from the bone structures.

The collagen fibres, on the other hand, appeared to be better preserved (even though in a state of decay) and were hardly present in the sub-periostal and sub-endostal lamellar, while in other areas they were still clearly appreciable as if the destructive process had had a double "attack site".

To confirm such a vision the discovery of a slight presence of collagen fibres in the lamellar in closet contact with the osteonic cavity and their conspicuous presence in the outermost cavities of the osteonic system.

Finally, it should be noted that the bone structure investigated using tomographic investigations was characterised by a notably reduced bone density, a large part of its inorganic constituents having been dispersed, differently to what occurs – after a similar amount of time–in the buried bone where generally a frequent interchange of inorganic materials with the burial earth can be seen, and where the CA/P ratio tends to remain constant [22].

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