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C. R. Palevol 3 (2004) 605–616
COMPTES RENDUS PALEVOL

General Paleontology (Palaeobiochemistry)

Fossilization of Haversian bone in aquatic environments

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Received 20 January 2004; accepted after revision 12 July 2004

Available online 30 September 2004

Written on invitation of the Editorial Board

Abstract

The process of fossilization is still in many respects poorly understood. Changing chemical conditions in the fossilizing bone allows us to distinguish successive stages of bone diagenesis. As long as organic compounds are present in the bone, the first stage, the early diagenesis, is not completed. However, the early diagenesis itself can be subdivided into three stages. Initially the organic compounds, mainly collagen, allow intensive microbial activity on the bone and this microbial decay characterizes the first stage by high decay rates and strongly reducing conditions. After about one year, the microbial activity ceases and the second stage begins, which lasts much longer and is characterized by chemical gelatinisation of collagen in the bone. Finally imbibition of the gelatinised collagen produces cracks across cement lines of secondary osteons and opens additional pathways for diffusion. In this last stage of the early diagenesis, collagen is replaced by apatite and other minerals. After this replacement, late diagenesis begins. The redox milieu in the fossilizing bone is now controlled by the environment, but the high phosphate content of the apatite still buffers the pH to high values. During late diagenesis, pH-dependent precipitation is the most important mineral formation process. ***To cite this article : H.-U. Pfretzschner, C. R. Palevol 3 (2004).***

Résumé

Fossilisation d'os haversiens en milieu aquatique. Le processus de fossilisation demeure encore mal compris à divers égards. Les conditions chimiques changeantes dans l'os en cours de fossilisation permettent de distinguer des étapes successives de la diagenèse osseuse. Tant que des composés organiques demeurent présents dans l'os, la première étape, ou diagenèse initiale, n'est pas complète. Cependant, on peut subdiviser cette première étape elle-même en trois stades. Initialement, les composants organiques, principalement le collagène, permettent une intense activité microbienne dans l'os, et cette dégradation bactérienne caractérise la première étape, du fait du taux élevé de la dégradation et des conditions réductrices. Après environ une année, l'activité microbienne cesse et la seconde étape débute ; elle dure bien plus longtemps et est caractérisée par la gélatinisation du collagène dans l'os. Finalement, l'imbibition du collagène gélatinisé induit des microfractures radiaires au niveau de la ligne cimentante des ostéones secondaires, ce qui ouvre des chemins supplémentaires pour la diffusion. Au cours de cette dernière étape du début de la diagenèse, le collagène est remplacé par de l'apatite et d'autres minéraux. Après ce remplacement, la diagenèse tardive commence. Le milieu redox de l'os en voie de fossilisation est à présent contrôlé par

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doi:10.1016/j.crpv.2004.07.006

l'environnement, mais la teneur élevée en phosphate de l'apatite maintient néanmoins le pH à des valeurs élevées. Pendant la diagenèse tardive, des précipitations dépendant du pH constituent le processus le plus important de formation des minéraux.
Pour citer cet article : H.-U. Pfretzschner, C. R. Palevol 3 (2004).

Keywords: Bone fossilization; Diagenesis; Decay; Collagen; Apatite; Mineral formation

Mots clés : Fossilisation de l'os ; Diagenèse ; Dégradation ; Collagène ; Apatite ; Formation minérale

1. Introduction

The process of bone fossilization is a very complex subject and many aspects are still not well understood. Fresh bone consists of 70 weight% of phosphatic minerals (60% hydroxyapatite and 10% other phosphatic minerals), 18 weight% of collagen (a protein), 9 weight% of water and 3% of other proteins, lipids and mucopolysaccharides [11]. During diagenesis, protein is more or less totally removed and replaced by inorganic substances. Additionally, the hydroxyapatite is altered by recrystallization [23,24,32,48,60,64] and by substitution of OH^- and PO_4^{3-} by Cl^-, F^- and CO_3^{2-} and it becomes a high carbonate fluorapatite (francolite) [24,45]. Additionally, Ca^{2+} in the apatite is substituted by metal ions, such as Fe^{2+}, U^{4+}, Zn^{2+}, Mn^{2+}, Sr^{2+}, Ba^{2+}, and others from the diagenetic fluids [1,9,25,30,35,38,39,46,47,51,61,64,65]. Hence the incorporation of uranium into the bone apatite is very common and most fossil bones possess considerable radioactivity [1,5,51,61]. Exchange of ions with the surrounding water does not only alter the elemental composition, but also the isotopic composition of the fossilizing bone [22,34,42,50,62]. The recrystallization of the apatite matrix transforms the fossil bone into a diagenetically more stable mineral phase and decreases the accessible surface of the crystals and therefore, from this time onwards, chemical reactions and substitutions of ions will take place with much slower rates. This gives a first hint to divide bone diagenesis into two major successive stages: the early diagenesis of bone deals with the decay processes of organic compounds in the bone, with the recrystallization and early ion exchange between surrounding water and the bone matrix. The high phosphate content of the bone together with the decay products of the bone protein characterizes the chemical milieu during early diagenesis [52]. This first stage ends with the replacement of the collagen by minerals. After this replacement is completed, the late diagenesis of the fossil bone starts [52,53,55]. Both, during early and during late diagenesis of bone, mineral formation can take place in the porosity of bone [15,32,52,54,55,56]. These minerals allow important clues about the pH and redox conditions during their formation.

2. Early diagenesis of bone

Early diagenetic processes in bone depend on the circumstances under which a bone is deposited. In soil bones are affected by fungi and bacteria, which destroy the internal structure [26,28,57] and, in arid climates, bones on the surface of the soil dry and get cracks. This paper will focus on the diagenetic processes in bones that are deposited under water, which is of special interest for palaeontological research.

The most important process during early diagenesis is the degradation of collagen. However, this process in decaying bone still is only roughly understood [17,19,20,21,23,27,29,41,66,67]. Results of previous studies revealed good indicators to estimate the degree of diagenetic degradation of collagen [20,21], such as infrared spectra or protein specific amino acid distributions, which became available by the application of new extraction methods, for example by application of collagenase. However, details of the degradation process are still under discussion. One of the key questions is whether the velocity of hydrolysis of the peptide bond differs between the different amino acids. Indeed some experimental results indicate a change of the amino acid spectra of collagen during experimental decay [66]. The knowledge of the mechanisms and kinetics of collagen degradation developed slowly [8,43,58,59] and data about collagen degradation in bone are rare [49,70]. Other researchers focused on to

the replacement of collagen by apatite and used scanning electron microscopy for this purpose [23,74]. More recently quantitative models of collagen hydrolysis and degradation velocities have become available [17,58]. The results of these models are in good agreement with the experimental data, such as measurements of the molecular mass distribution in fragments of degraded collagen [31]. Furthermore these models identified problems of classical experimental methods. For example, there should occur an increase of N-terminated protein chains in the insoluble fraction during protein degradation, which would in turn increase the rate of racemization [17]. Furthermore small peptides, which are not stabilized by hydrogen bonds, should be faster split off the insoluble fraction. As these peptides are too small to be detected, immunological tests underestimate the total protein amount [17]. Another problem is the racemization process itself. As racemization of certain amino acids is frequently used as an indicator for diagenetic alteration of bone samples (e.g., [44,72]) a quantitative understanding of this process would be of great interest. However, experimental data are somewhat confusing. Whereas estimates of racemization rates in fossils show only little variation between the different amino acids (with exception of aspartic acid) experimental studies indicate marked differences in rates of hydrolysis of peptid bonds and racemization [16]. Obviously the details of the processes of hydrolysis and racemization are still not well understood [2,3,16]. During decay, different amino acids show different rates of loss [68] due to different rates of hydrolysis as well as to additional degradation reactions, such as non-enzymatic deamination, decarboxylation, ester formation and the Maillard reaction, all of which transform certain amino acids into derivatives [4,12,33,63,68,73]. Hydrolysis and racemization of aspartic acid are of special interest, as the D/L-ratio of aspartic acid is used as a kind of chemical chronometer as well as an indicator for the degree of depurination of ancient DNA in fossil bone samples [67]. Recent quantitative models, which deal with aspartic-acid racemization kinetics [18], indicate that the racemization kinetics of this amino acid highly depends on the conformation of the collagen molecule and the D/L -ratio reflects the ratio of non-helical to helical collagen molecules. This example once more shows the problems of 'black-box'-approaches, which use a single indicator substance to measure the degree of diagenesis of bone collagen. Obviously much more detailed knowledge about the mechanisms of collagen degradation is needed, but already with a black box approach several important aspects can be discussed.

To understand the processes of mineral formation during the early diagenesis, the chemical milieu in decaying bone must be known. In this respect, bone histology must be reconsidered. Most types of bone are very dense and external reactants can enter the interior of the bone only by diffusion. Fluid flow through the narrow canals in osteons as well as through the tiny canaliculi does not occur. Especially in the Haversian bone, the pathways for diffusion are very long. The reason is that usually Haversian canals running parallel to the long axis of a long bone and Volkmann's canals, which connect adjacent Haversian canals, are rare. Furthermore, secondary osteons, which build up the Haversian bone, are surrounded by a mineral wall. This circumferential mineral wall is known in cross sections of osteons as the 'cement line'. Therefore diffusion, starting from a Haversian canal, cannot get beyond the cement line of an osteon. The regions beyond can only be reached via the Haversian canal, a Volkmann's canal, back again the adjacent Haversian canal and finally via the canaliculi of the adjacent osteon. As a result, diffusion in bone is a very slow process. Measurements of effective diffusion constants in fresh bones with different stains yielded values of about $D_{eff} = 0.02$ mm^2/day, which are about 500 times lower, than in free water (D = 11 mm^2 d^{-1}) [52]. Consequently, the external influence on the chemical milieu in decaying bone is very low and exchange processes between bone and the surrounding water is very slow in early diagenesis.

Due to the composition of bone, the chemical milieu is characterized by high pH values, between 8 and 10, and by a very low redox potential (lower than –200 mV) [52]. Firstly, the high phosphate content of bone minerals buffers the pH to high values. Measurements of the solubility of hydroxyapatite in water using conductimetry and spectral photometry as well as thermodynamic data allow the calculation of the equilibrium pH value in solutions that are in contact with solid hydroxyapatite [52]. These calculations yield equilibrium pH values between 8 and 10 [52] and this is in good agreement with pH measurements in laboratory experiments on decaying bone samples.

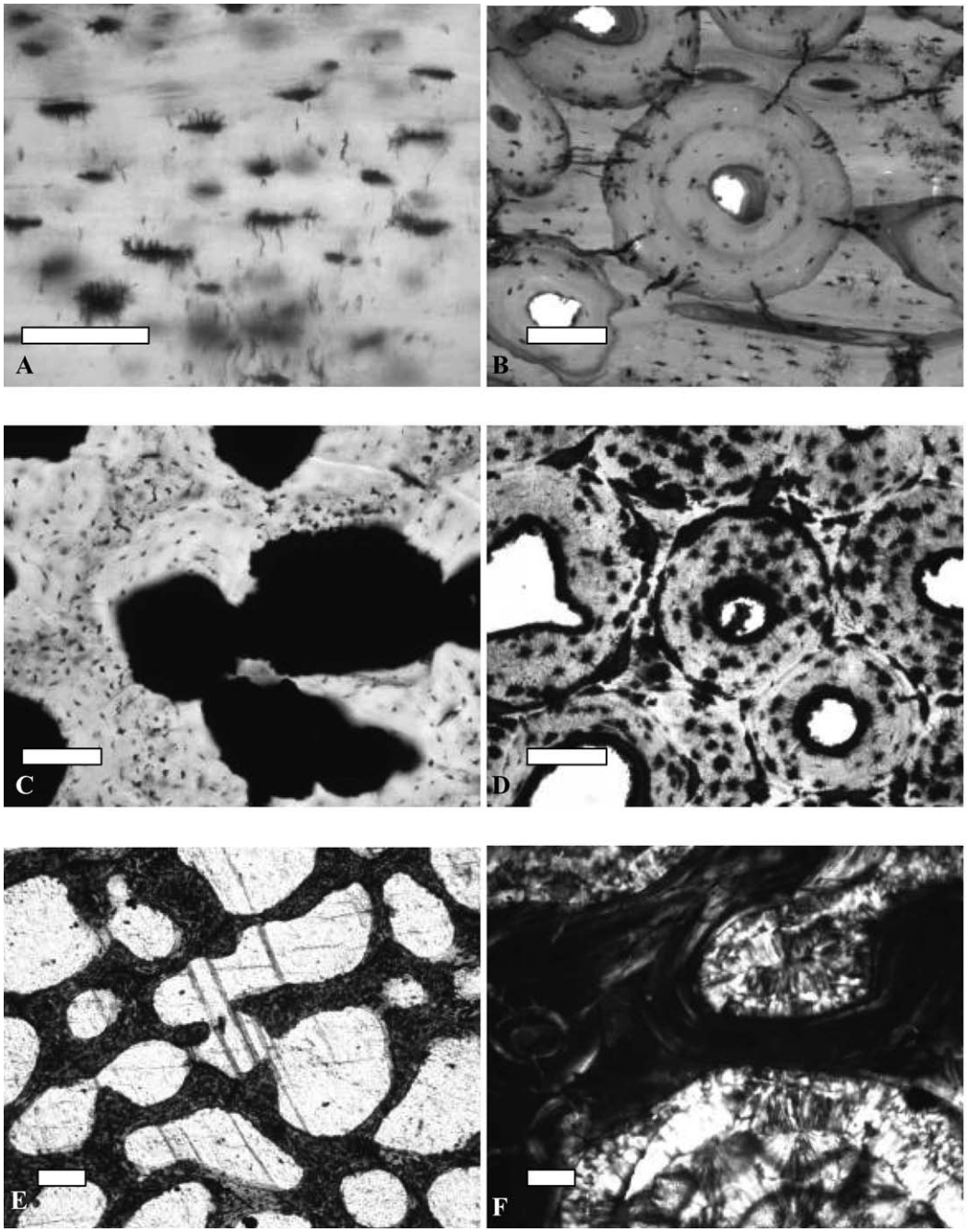

Fig. 1. (**A**) Early diagenetic pyrite formation by sulphide precipitation in osteocyte lacunae. *Equus*, metatarsal, Pleistocene, Rhine gravel. Scale bar: 50 µm. (**B**) Radial microcracks. Longbone of *Camarasaurus*, CMNH 36021. Scale bar: 100 µm. (**C**) Late diagenetic pyrite formation by pH-dependent precipitation. Extensive replacement of bone matrix by pyrite. Whale bone, indet., Miocene, Antwerp. Scale bar 100 µm. (**D**) Hematite fillings formed by pH-dependent precipitation of ferrous hydroxide and transformation into hematite. Extensive replacement of bone matrix by hematite. Tibia of *Barosaurus*, Tendaguru Fm., Africa. Scale bar: 100 µm. (**E**) Late diagenetic calcite formation in the porosity of trabecular bone. *Stenopterygius* vertebra, Posidonian shale, Dotternhausen, Germany. Scale bar: 100 µm. (**F**) Late diagenetic silica filling of the porosity of trabecular bone. No replacement of the bone matrix by silica. Dinosaur indet., Morrison Fm., Utah. Scale bar: 100 µm.

The pH was measured in the surrounding water and ranged between 8 and 8.5 [52]. Besides the buffering effect of the phosphate, the release of ammonia during the decay of collagen also raises the pH [52]. The degradation of collagen is partly due to non-biotic leaching and partly due to microbial activity on the bone surface. Typically in experiments on bone diagenesis in freshwater, the redox potential sinks very fast (from some hours to one or two days, depending on the boundary conditions) to values of about –200 mV and remains low during several months [52]. During this time, oxygen and all other oxidants (nitrate, nitrite, ferric iron Fe^{3+} and others) are depleted in the surrounding solution, even if oxygen from the air has free access to the water in which the bone sample was stored. In long-term experiments, microbial activity fades after about six months (under laboratory conditions at +20 °C) and then the redox potential of the water slowly begins to raise again to values about –50 to 0 mV [52]. Daily measurements of the amount of inorganic nitrogen compounds (nitrate, nitrite and ammonia) that were released from the bone samples allow us to model the decay rate of collagen throughout the first year. Whereas in large bones (for example human long bones or larger), after one year, only 5 to 15% of the collagen is destroyed, in small rodent bones the total protein content has vanished during this time. These low values of protein loss due to microbial activity correspond very well to the microscopic appearance of bone samples. Collagen had vanished throughout a one-year experiment with bone samples of 1-cm diameter (compact bone) down to a depth of 20–30 μm below the surface.

Even if there is no measurement available at present, it can be easily imagined that the chemical milieu in compact bone still is characterized by reducing conditions and high pH after microbial acitivity on the surface has ceased. During the next years decay in the bone will be mainly due to abiotic processes. Therefore, after an initial biotic phase of decay, a second abiotic phase follows in the early diagenesis. During this time, collagen will be gelatinised by successive splitting into shorter protein chains [17]. This gelatinisation process is pH dependent and is considerably faster under high pH conditions than in neutral water. Increasing gelatinisation of the collagen allows more and more hydration of the protein. Whereas fresh collagen contains only small amounts of water [71], hydration of gelatinised collagen swells considerably the bone sample. This swelling causes a characteristic pattern of microcracks in the Haversian bone [53]. In a secondary osteon, hydration of the gelatinised collagen is due to water supply via the Haversian canal and canaliculi. Therefore, the bone material in a secondary osteon swells faster than the regions beyond its circumference. This causes tension stress in the mineral sheet, which surrounds each secondary osteon. Finally, after reaching a critical stress, the mineral sheet cracks. In cross sections of a fossil Haversian bone, which fossilized under water, these cracks can be seen as short radial cracks at the circumference of each secondary osteon (Fig. 1B). Recently these cracks were produced experimentally. Thin sections of fresh bone were studied in a high-pH solution under the microscope. After 5 to 10 h the characteristic radial cracks appeared (Pfretzschner, in prep.). These radial cracks are very important for the further diagenesis, as they open straight and short pathways through the whole bone volume, which increases the exchange processes with the surroundings of the bone. This means that decay and leaching of the already gelatinised collagen continue and that the exchange of ions between the water and the bone mineral is enhanced. Estimates of the importance of these new pathways are possible on the basis of the percolation theory. The results from fossilized bone clearly show that the radial cracks considerably accelerate the fossilization process of the Haversian bone [53]. This third and last phase of early

Fig. 1. (**A**) Formation de pyrite en début de diagenèse par précipitation de sulfure dans des logettes périostéocytaires. *Equus*, métatarsien, Pléistocène, gravière du Rhin. Échelle = 50 μm. (**B**) Microfractures radiales en périphérie d'ostéones secondaires. Os long de *Camarasaurus*, CMNH 36021. Échelle = 100 μm. (**C**) Formation de pyrite en cours de diagenèse tardive par précipitation contrôlée par le pH. Remplacement étendu de la matrice osseuse par de la pyrite. Os indéterminé, Miocène, Anvers. Échelle = 100 μm. (**D**) Remplissage hématitique produit par la précipitation dépendant du pH d'hydroxyde ferreux transformé en hématite. La matrice osseuse est largement remplacée par l'hématite. Tibia de *Barosaurus*, Tendaguru, Afrique orientale (Tanzanie). Échelle = 100 μm. (**E**) Formation diagénétique tardive de calcite dans les cavités de l'os spongieux. Vertèbre de *Stenopterygius*, marne à posidonies, Dotternhausen, Allemagne. Échelle = 100 μm. (**F**) Formation diagénétique tardive de silice dans les cavités d'os trabéculaire. II n'y a pas eu de remplacement de la matrice osseuse par la silice. Dinosaure indéterminé, formation de Morrison, Utah, USA. Échelle = 100 μm.

diagenesis is characterized by the appearance of the radial cracks in secondary osteons, high exchange rates, high leaching rates and an increasing influence of the external chemical milieu. Indeed, during this phase, apatite in the bone possesses a huge surface due to the tiny dimensions of the crystals and exchange of ions with the surrounding water is very strong. Calcium is partly replaced in the crystal lattice by Fe^{2+}, Mn^{2+}, Zn^{2+}, U^{4+}, Sr^{2+}, Na^{+} and others, and phosphate is replaced by carbonate, fluoride and chloride. Replacement of phosphate by arsenate and incorporation of UO_2^{2+} seems not very probable during this phase, as by the presence of organic material the redox conditions in bone should still be strongly reducing. Additionally to the replacement, external ions are also just bound to the surface. Probably it is this final stage of the early diagenesis, when a recrystallization of the bone apatite happens. Fossil bones usually do show a higher apatite crystallinity than fresh bone samples [50,60,62,68] and the hydroxyapatite has changed into a high-carbonate apatite (francolite), which also shows an increased fluoride content [24,32,45,64]. Depending on the phosphate supply from the surrounding water epitaxial growth on the primary apatite, crystallites may occur or the crystallites may be dissolved and precipitated as a colloid again. In the latter case, the orientation of the *c*-axis is lost and bone is preserved in a microcrystalline apatite variation, which was formerly called collophan. This apatite variation does not show any optical activity under the polarizing microscope. However, the details of the replacement of collagen by minerals are still unknown. The process of early diagenesis described so far makes clear that phosphate from the soft tissues of the individual, such as ATP in muscles, plays no role during the recrystallization of the bone apatite. So far it is unknown how much phosphate and calcium is required from the environment to build a stable fossil bone. During recrystallization of the bone apatite, small cavities, such as the canaliculi, are often filled with apatite. Therefore, after the complete replacement of collagen by apatite, the osteocyte lacunae are usually well isolated from external reactants.

With the loss of the organic content and its replacement by minerals, early diagenesis ends and late diagenesis starts. Application of the RGT-rule (= increase of reaction temperature for 10 °C doubles the reaction rate) to the results of thermal degradation experiments [13] gives a rough idea about how fast collagen degradation actually is. Another rough estimate of the time span of the early diagenesis is to extrapolate the time needed for the formation of radial cracks by gelatinisation from experiments under high-pH conditions to natural conditions. Both estimates are consistent and very roughly yield 50 to 500 years for the endurance of early diagenesis under natural conditions.

However, these estimates exclude certain circumstances. As it is well known, many bones from Pleistocene deposits still possess a large amount of collagen. This is probably due to some organic substances from the environment, which have a tanning effect on the collagen [17,69]. As collagen hydrolysis slows down much more under cold conditions than the tanning effect of these reactants, collagen remains can be preserved until the present days in these bones. These bones usually have lost all collagen superficially up to some few millimetres depth, but the bulk of compact bone still contains most of the collagen [54]. Hence these bones have entered already late diagenesis in the superficial regions, but remain still in the early diagenesis in the centre.

3. Pyrite formation during early diagenesis

Minerals precipitated during early diagenesis occur only in those cavities in bone, which are accessible at that time, such as Haversian canals, osteocyte lacunae and the proximal parts of canaliculi (Fig. 1A). Later, towards the end of early diagenesis, minerals can be precipitated also in the radial cracks and along the cement line of secondary osteons [53–55]. During early diagenesis, the chemical milieu in decaying bone is characterized by high pH values and low redox conditions. Similar conditions also can be expected in the vicinity of the decaying bone. Mobile ferric iron (Fe^{3+}) approaching the bone will be reduced into ferrous iron (Fe^{2+}). Furthermore decaying collagen will release sulphide into the water. Under these conditions iron sulphide (FeS) is precipitated as follows [54,55]:

$$Fe^{2+} + H_2S \rightarrow \underline{FeS}\downarrow + 2\ H^2 \quad (1)$$

As this precipitation requires a low redox potential (at pH 8, $E_h < -230$ mV) and the presence of sulphide in the aqueous solution, it is restricted to early diagenesis. The precipitated iron sulphide FeS is later trans-

ferred into pyrite (FeS_2). This precipitation just fills up the cavities in the bone without damage to the bone wall and the resulting pyrite usually forms precise casts of the histological structures (Fig. 1A). However, the pyrite fillings also block the pathways through the bone and so decrease the accessibility of ferrous iron, which can enter the bone only by diffusion. The result is often a patchy distribution of pyrite in fossil bone. Early diagenetic pyrite usually occurs in osteocyte lacunae and in the proximal parts of the canaliculi. Due to the slow diffusion through fresh bone the access of iron in the bone is very restricted. Therefore, the total amount of precipitated pyrite is small and pyrite fillings of osteocyte lacunae usually are hollow casts [55]. In Haversian canals, early diagenetic pyrite may occur also, but due to the larger volume in usually it does not fill the whole cross section. Under limnic conditions, early diagenetic pyrite formation in the sediment is usually controlled by the amount of sulphide, as iron usually is available in excess. This is certainly also true for the surface of the bone. In the compact bone, however, decaying protein releases during early diagenesis a lot of sulphide and the access of iron is restricted by low diffusion constants. At that time pyrite formation in canaliculi and osteocyte lacunae should be iron-controlled. Later, when sulphide production ceases and diagenetic cracks appear pyrite formation may be sulphide-controlled. If the bone is deposited in seawater, additional sulphate ($SO_4^{2-}SO_4^{2-}$) will be reduced to sulphide (S^{2-}), due to the high natural concentration of sulphate ($c_{\text{sulphate}} = 2.65\ \text{g kg}^{-1}{}_{\text{sea water}}$). Therefore pyrite is very common in fossil bones from marine deposits and often the pyrite content is considerably higher than in bones from limnic sediments. These high pyrite contents usually result in a black or dark brown colour of the fossil bones. Under these conditions, supply with sulphide is high and the precipitation is controlled by iron.

4. Late diagenesis

During late diagenesis, the conditions for chemical reactions in fossil bone are quite different from the conditions in the early diagenesis and most rates of the chemical reactions are much slower. Usually all organic remains have vanished at the beginning of the late diagenesis, except for some traces, which may persist in the apatite matrix [20]. Consequently, the redox conditions are no longer controlled by the bone itself, but by the environmental conditions. On the other hand, the pH is still buffered to high values by phosphate from the high apatite content in the bone. Therefore, pH-dependent precipitation is the most important mineral formation process in late diagenesis. Metal ions in aqueous solutions, which come into contact with the fossil bone or enter it by diffusion will meet high pH conditions and precipitate as hydroxides.

$$Me^{n+} + n\ OH^- \rightarrow \underline{Me(OH)_n}\downarrow \quad (2)$$

As this precipitation reduces the OH^-concentration and lowers the pH, a small amount of apatite will dissolve and the phosphate buffers again the pH to the high equilibrium value: PO

$$PO_4^{3-} + 3\ H_2O \rightleftharpoons HPO_4^{2-} + OH^- + 2\ H_2O \rightleftharpoons H_2PO_4^{2-} + 2\ OH^- + H_2O \rightleftharpoons H_3PO_4 + 3\ OH^- \quad (3)$$

Consequently, pH-dependent precipitation of minerals is always connected with etching of the surface of the fossil bone or of the cavities in the bone. These minerals always replace a certain amount of apatite during their formation and this characteristic can be used to identify them under the microscope (Fig. 1C and D). Furthermore, the minerals that are formed during late diagenesis occur only in cavities, which are still accessible to external fluids, such as Haversian canals or late diagenetic cracks, which open by tectonic pressure and run straight through the bone sample. Osteocyte lacunae and canaliculi usually lack late diagenetic minerals. However, in some rare cases the canaliculi and lacunae are not blocked by apatite or pyrite and pH-dependent precipitation can occur there too. In contrast to the pyrite formation in the early diagenesis, which perfectly casts the histological structures, the pH-dependent precipitation destroys more or less histological structures by etching.

Many oxides in fossil bone are precipitated initially as hydroxides by the high pH in late diagenesis. Ferrous iron can be precipitated from aqueous solutions as $Fe(OH)_2$,which is later transformed to hematite (Fe_2O_3) or goethite (FeOOH) (Fig. 1D). Bones, which fossilized under well-oxygenated conditions in shallow water at the sea shore or in hot and arid climates in

river beds often show a characteristic red-coloured hematite coating and hematite fillings of Haversian canals. Goethite is another oxic iron mineral, which frequently occurs in fossil bones [24,32,56]. Manganese oxides, such as pyrolusite (MnO_2) are also precipitated initially as hydroxide (Pfretzschner and Tütken, in prep.). Due to the higher concentration of manganese in rivers and in groundwater, pyrolusite mainly occurs in bones from terrestrial deposits and is usually absent in bones from marine deposits. The mobile Mn^{2+} from the groundwater enters the bone and initially $Mn(OH)_2$ is precipitated, which transforms by loss of water first into MnO and later into pyrolusite (MnO_2).

Besides hydroxides and oxides, carbonates also can be precipitated by the raised pH in the bone as the hydrogencarbonate–carbonate equilibrium is shifted to the carbonate side:

$$HCO_3^- + OH^- \rightleftharpoons H_2O + CO_3^{2-} \quad (4)$$

In bones calcite usually occurs as a typical late diagenetic filling of Haversian canals, tectonic cracks and the cavities in trabecular bone [32]. Other carbonates (siderite, kutnahorite and others) also occasionally occur in fossil bones [15] (Fig. 1E).

Last but not least, even pyrite is often precipitated during late diagenesis by high pH values [55]. Late diagenetic pyrite can be distinguished from early diagenetic pyrite by the characteristics of the pH-precipitated minerals listed above. It usually shows replacement of the adjacent bone and occurs mainly in Haversian canals and in tectonic cracks in the bone [40,55] (Fig. 1C). To precipitate FeS, a solution saturated with iron and sulphide must have access to the fossil bone. Such conditions are usually found in marine sediments below the RPD [6,7,10,14,37]. Indeed late diagenetic pyrite can frequently be observed in bones from marine deposits. Only this late diagenetic pyrite indicates strongly reducing external conditions.

Finally, silica should be mentioned as a mineral, which frequently fills Haversian canals, tectonic cracks and intertrabecular cavities [32] (Fig. 1F). However, the process of silicification is not due to changing pH conditions, but seems to have something to do with recrystallization of the silica [36]. Consequently, silicification usually does not affect the bone and preserves well the histological structures. Silicified bones usually occur in terrestrial deposits, where they fossilized near the surface under warm and dry conditions. The same process, which enriches silica in the superficial soil, can also lead to silicification of bones. Volcanic activity is not a necessary requirement for the silicification of bones.

Later, during late diagenesis, external oxidants can enter the fossil bone and transform the existing minerals. Often pyrite, which was formed earlier during diagenesis, is later oxidized and transformed into hematite [56]. In those cases, small remains of original pyrite can usually be found in the centre of the hematite. As oxidation of pyrite produces sulphuric acid:

$$2\ FeS_2 + 7.5\ O_2 + 7\ H_2O \rightarrow 2\ Fe(OH)_3 + 4\ H_2SO_4 \quad (5)$$

the adjacent bone matrix is etched and secondary replacement of apatite by iron oxides can be observed under the microscope.

5. Conclusion

Fossilization of the Haversian bone can be subdivided into several successive stages by the characteristics of the chemical milieu in the bone (Fig. 2). As a first step, a principal division into early and late diagenesis seems reasonable. Whereas the chemical milieu during the early diagenesis is controlled by the presence of decaying organic compounds and the presence of phosphate (apatite), in the late diagenesis, it is the presence of apatite alone that characterizes pH conditions. The redox conditions are controlled by environmental factors. Due to the chemical milieu in the bone, the mineral formation processes differ between the two stages. During early diagenesis, sulphide precipitation is a very important reaction, whereas during late diagenesis, most minerals are precipitated due to the high pH values. Furthermore the decay and mineral formation rates differ in both stages. Late diagenetic reactions usually do show much slower rates of mineral formation.

In a second step, the early diagenesis can be divided into three steps: an initial one, which is characterized by intensive microbial activity, a longer-lasting second step, without any microbial activity, but chemical decay of collagen and finally a third step, during which

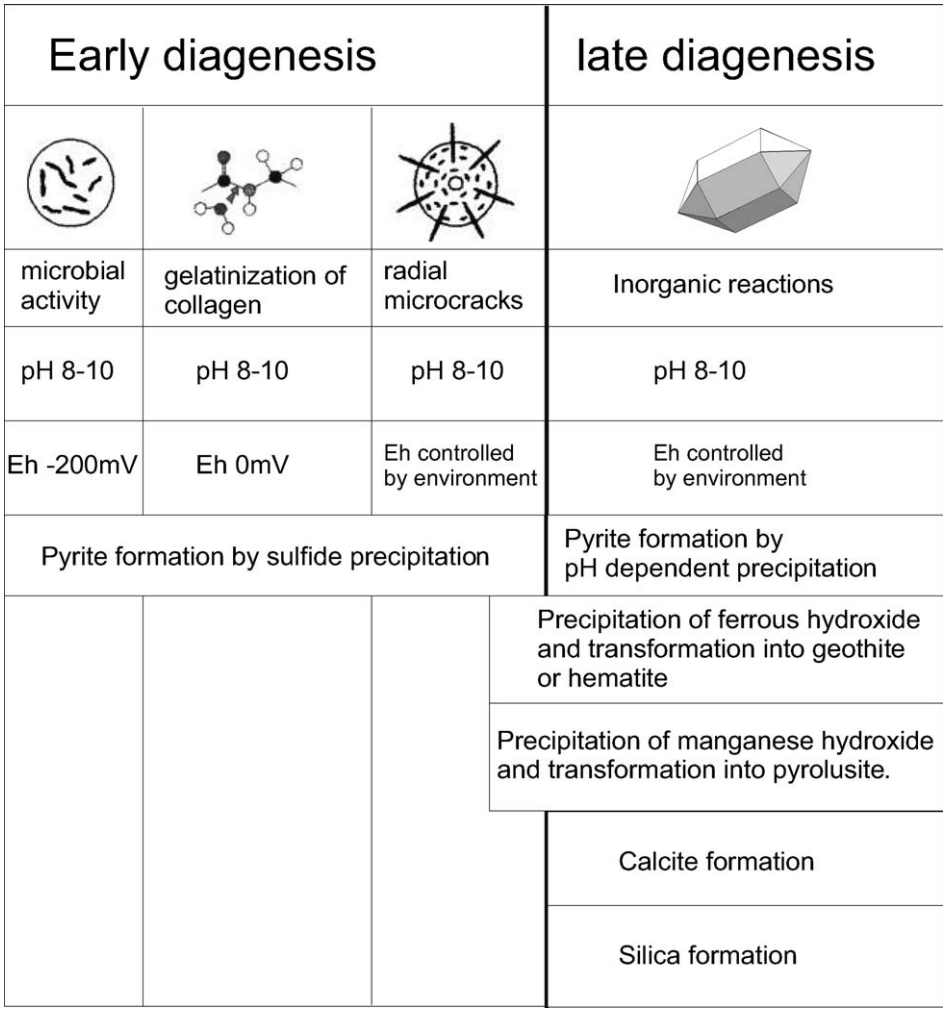

Fig. 2. Schematic overview of the successive stages in bone fossilization, the chemical conditions in the bone and the main minerals that may occur in fossil bone. Detailed discussion.
Fig. 2. Schéma général des étapes successives de la fossilisation de l'os et les principaux minéraux susceptibles d'être déposés lors des diverses étapes. Discussion détaillée.

the replacement of the gelatinised collagen by apatite and other minerals takes place.

A deeper understanding of the diagenetic processes during bone fossilization still requires a lot of research. Especially the replacement of collagen by minerals is still very poorly understood. On the other hand, a better understanding of fossilization processes is basically important for the search of ancient DNA or for the interpretation of stable isotopes. It seems also of central interest for the palaeohistological research. Together with sedimentological research the interpretation of the diagenesis of fossil bones may also help in the reconstruction of the palaeoenvironment. All together, bone diagenesis seems to be helpful for several palaeontological and geological research fields and further intensive research on this interdisciplinary field seems desirable.

Acknowledgements

I want to thank the Deutsche Forschungsgemeinschaft, which made this research project possible through its financial support. Furthermore, I want to

thank Prof. W. v. Koenigswald (Bonn), Dr. R. Wild (Stuttgart), Dr. D. Berman (Pittsburgh) and Prof. S. Ge (Changchun), who made the material available to me. For reading and commenting the manuscript, I thank Prof. Dr. W.-E. Reif (Tübingen).

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