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New data on bone matrix and its proteins

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Abstract

After a synthesis on some recent data on the biology of bone tissues senso lato, this paper focuses on the limits of known variability of bone regarding its amount of mineralisation (maximal and minimal) and on the underlying control mechanisms. Two models of hypermineralised bones are reviewed among cetaceans: the rostrum of the 'beaked whale' *Mesoplodon* and the tympanic bulla of the dolphin *Delphinus*. In the first case, hypermineralisation is reached lately through an ultrastructural specialization of the secondary osteons. In the second case, hypermineralisation starts at once, during foetal life, through a specialization of the primary osteons. In both cases, ultrastructural peculiarities of collagen fibrillogenesis are demonstrated. Diameter and spacing of the fibrils leave an exceptional empty space available for mineral deposition. Conversely, hypomineralisation is observed on the model of the Teleost scale. The basal plate of the scale is made of a regular 'biological plywood' of densely packed collageneous fibres. The mineral is laid down mostly in the interfibrillary spaces. All such tissue modulations seem to be rigorously controlled by details of the osteoblast biosynthetic activities. In a given situation, osteoblasts may express only a subset of molecules from their potential complete biosynthetic repertoire. The debate now centres on how osteoblasts acquire positional information to express this subset. ***To cite this article: L. Zylberberg, C. R. Palevol 3 (2004)***.

Résumé

Données récentes sur les constituants protéiques de la matrice osseuse. Après une synthèse de certaines acquisitions récentes sur la biologie du tissu osseux sensu lato, on examine les limites de variabilité connues de ce tissu en ce qui concerne son degré de minéralisation (maximal et minimal) et les mécanismes de contrôle de celui-ci. Deux modèles d'os hyperminéralisé sont examinés chez les Cétacés : le rostre de la « baleine à bec » *Mesoplodon* et la bulle tympanique du dauphin *Delphinus*. Dans le premier cas, l'hyperminéralisation est acquise tardivement, du fait d'une spécialisation ultrastructurale des ostéones secondaires. Dans le second cas, l'hyperminéralisation est acquise d'emblée, du fait spécialisation de l'ultrastructure des ostéones primaires dès le stade fœtal. Dans les deux cas, des particularités ultra-structurales de la fibrillogenèse collagénique sont mises en évidence, le calibre et l'agencement des fibrilles laissant un espace disponible, exceptionnellement élevé, pour le dépôt minéral. L'hypominéralisation, à l'inverse, est observée sur le modèle écaille des Téléostéens. La plaque basale de l'écaille est constituée d'un contreplaqué biologique très régulier, formé de paquets de fibrilles collagéniques. Le biominéral déposé occupe

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plutôt les espaces interfibrillaires. Toutes ces modulations tissulaires semblent rigoureusement contrôlées par le détail de l'activité biosynthétique des ostéoblastes. Ceux-ci ne semblent exprimer, dans un contexte donné, qu'une portion limitée des molécules faisant partie du registre biosynthétique potentiel complet de ces cellules. Le débat tourne à présent sur la compréhension des mécanismes permettant aux ostéoblastes d'acquérir l'information de position liée à l'expression de telle ou telle partie de leur registre. ***Pour citer cet article : L. Zylberberg, C. R. Palevol 3 (2004)***.

Keywords: Collagen; Bone; Hypermineralisation; Hypomineralisation; Osteoblast

Mots clés: Collagène; Os; Hyperminéralisation; Hypominéralisation; Ostéoblaste

1. General considerations on bone structure and composition

The skeleton is a complex organ composed of bones whose size, shape, number and arrangement distinguish vertebrate species from one another. Its development is a highly regulated process. Skeletal elements derive from mesenchymal cells that superficially differentiate directly into osteoblasts in a process called membranous ossification or into chondroblasts forming a cartilage template that is subsequently replaced by bone, where osteoblasts are brought by vascular invasion. This process of bone formation is called endochondral ossification. Bone tissue, whatever its ontogenesis is, is a composite material within which collagens polymerised into fibrils constitute a preformed organic lattice for the mineral deposit. The fibrillar collagens are responsible for the tensile strength of the bone and counterbalance the brittleness of the mineral that consists of a calcium phosphate belonging to the family of apatites [9]. Bones show a variety of structural organisations that are related to the balance between the amount of collagen and mineral [29] and to which are linked a wide spectrum of mechanical properties [12]. These mechanical properties of bones also depend on the spatial arrangement of the collagen fibrils that are the basic organisational motif of the bone matrix [48]. Collagen fibrils are either organised in a loose network and they form a woven bone matrix or they are closely packed and approximately parallel to each other with the same orientation forming a parallel-fibered bone matrix. Both tissues can be organised to form a compact bone or a cancellous bone (= spongy bone). The distinction between these two types of bone is related to their porosity. Compact bone shows a higher volume of bone tissue than other soft tissues; conversely, cancellous bone presents trabeculae surrounding a greater volume of soft tissues (cf. [17] for the typology of bone tissue).

Primary bone corresponds to bone formed where antecedent bone tissue does not exist. Once formed, primary bone is submitted to a more or less important remodelling related not only to morphogenesis but also to meet the varied functional demands. Bone remodelling involves the dual processes of resorption and redeposition of a de novo formed bone [18], the secondary bone, which corresponds to a bone tissue deposited where an antecedent bone tissue has been resorbed [17] (Fig. 1). The diversity of bone tissues is found in primary as well as in secondary bone.

In all bone tissues, osteoblasts regulate the deposition of the bone matrix components and ensure the formation and the deposition of the mineral phase (inter alia, [20,42,51]). They can be distinguished from fibroblasts by genes that control their differentiation and proliferation. *Runx2 (Cbfa1)* [14,15,28] and *osterix* [34] control osteoblast differentiation. *Runx2* is necessary and sufficient for osteoblast differentiation and it is the earliest and most specific marker of the osteoblast lineage [16]. Temporal control of *Runx2* activity is important not only for bone development but also for bone maintenance in adults [15,19]. *Runx2* was shown to interact with the promoter region of the *osteocalcin* gene; osteocalcin is a highly specific osteoblastic marker produced during bone formation [24]. The LRP5 (low-density lipoprotein receptor-related protein 5) signalling pathway controls osteoblast proliferation [23].

Osteoblasts produce two types of proteins found in bone extracellular matrix (ECM): the collagens mostly type-I collagen (90% of the bone matrix proteins [39]) and non-collagenous proteins. Type-I collagen, the

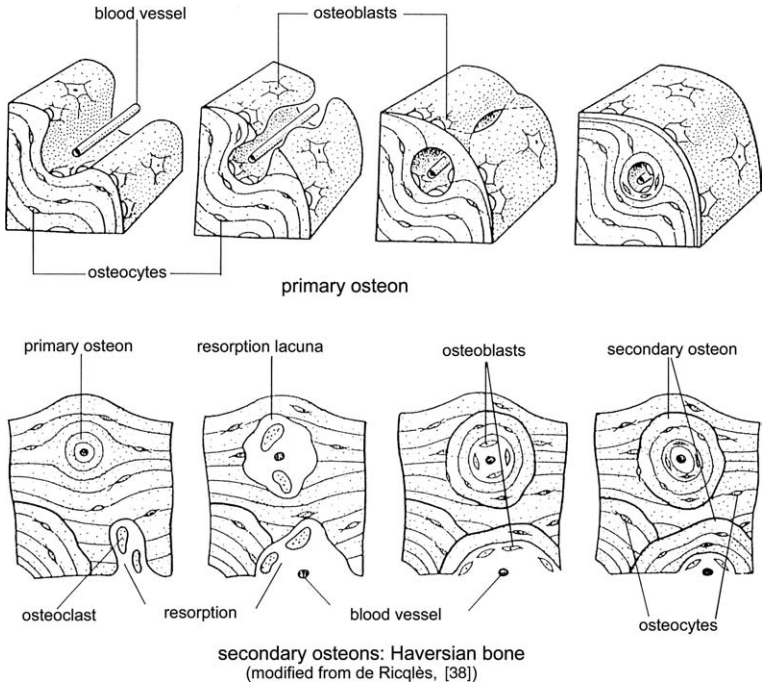


Fig. 1. Diagram of the formation of primary and secondary osteons.
Fig. 1. Schéma de la formation des ostéones primaires et secondaires.

predominant protein of the bone ECM, has been considered as the primary gene product of the osteoblasts [21]. Like other collagen types, the main characteristic of type-I collagen is its triple helical domains (reviewed by [40,46]). Each molecule of type-I collagen is composed of two α1 chains and one α2 chain [$\alpha1(I)_2$ – α2(I)]. The α chains are encoded by two different genes located on two different chromosomes but showing a very similar structure. The α chains are secreted as propeptides; then the C- and N- terminal propeptides are rapidly cleaved by N-and C-proteinases respectively [37]. The C- and N-telopeptides that remain attached to the triple helix play an important role as sites of cross-links [46]. Cross-links are created by a posttranslational processing that is responsible for minor but significant differences between soft- and hard-tissue type-I collagens. These differences reflect the degree of lysyl hydroxylation, the extent and type of hydroxylysyl glycosylation and the distribution of intra- and inter molecular covalent cross-links that increase the tensile strength of the fibrils [40]. Indeed, posttranslational modifications involved in fibrillogenesis are thought to regulate the diameter of the collagen fibrils [46] that is important in subsequent mineralisation processes [26,30,45,49]. In the extracellular matrix, the collagen molecules aggregate in a highly specific fashion that gives rise to collagen fibrils showing the axial banding pattern observed in electron microscopy.

Besides the most abundant fibrillar type-I collagen, bone contains the minor fibrillar type-V collagen, both type I and type V being co-distributed within the same fibril [3]. They interact as heterotypic fibrils [2]. An increasing amount of type-V collagen leads to a decrease in the diameter of the heterotypic fibril [3,32]. Type V may play a role in the mineralising matrix, but it appears to have little effect on the tissues once mineralised [47].

However, very few studies on the cellular control of collagen synthesis were carried out in vivo because they are hampered by the fact that some factors would either influence directly collagen synthesis or act indirectly by modifying the production of other factors that will themselves affect collagen synthesis.

The non-collagenous proteins including osteocalcin that occurs at the highest molar concentration of all non-collagenous proteins found in the ECM [24] are osteonectin, osteospontin, and bone sialo proteins. Be-

sides proteins, the ECM contains proteoglycans including versican, decorin, biglycan, fibromodulin, osteoadherin, osteoglycin [39].

The osteoblast activity can also be modified by different local factors such as bone morphogenetic proteins (BMP), transforming growth factors (TGF-β), insulin-like growth factors I (IFG-I) and insulin-like growth factor II (IFG-II) that can stimulate type-I collagen production. The tumour necrosis factor α (TNF α), a cytokine, stimulates osteoblast proliferation, but inhibits the production of bone extracellular matrix components, increases collagenase production and thus extracellular matrix degradation [22]. Other cytokines such as interleukin and interferon γ are known to modulate type-I collagen production [40].

These locally produced factors are joined by systemic factors such as vitamin D, glucocorticoids, hormones including growth hormone, thyroid hormone, parathyroid hormone, oestrogens, and recently leptin, a hormone involved in the control of body weight was shown to regulate bone formation via the sympathetic nervous system [43].

Important data on the bone formation have come from studies on genetically defined mouse models. In this field of research, most of the studies rely on the relationships between structure and mechanical properties of long bones, especially in mammals.

Mechanical loads control the local structural adaptations [12]. The mediators for this control include direct action on cellular stress-sensitive calcium channels and also integrins and paracrins mediators such as prostaglandins E_2, (PEG_2), prostacyclin and nitric oxide [36]. The rate of bone matrix deposition by differentiated osteoblasts is regulated by the gene *Runx2 (Runx2)* that acts as a maintenance factor. (Cbfa1) *Runx2* is at the top of a genetic cascade controlling its own expression and the expression of the major osteoblast genes including type-I collagen genes [14].

However, what remain to be identified are the local factors controlling specific bone structure such as hypermineralised bone that differs from a regular bone.

2. Dense bones with a very high mineral content

The most highly mineralised bones were described in marine mammals. The rostrum of the beaked whale

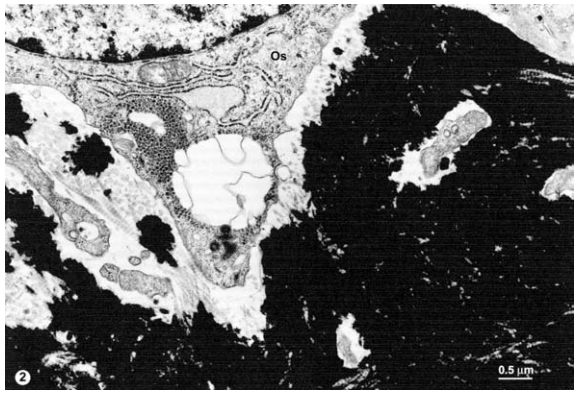


Fig. 2. TEM. Section a 'regular bone'. The osteoblast (Os) is equipped with long cytoplasmic processes penetrating within the mineralised ECM. The banding of the collagen fibrils is not totally obscured by the mineral deposit.
Fig. 2. Coupe de tissu « normal » vue en microscopie électronique à transmission. L'ostéoblaste (os) est pourvu de longs processus cytoplasmiques pénétrant la matrice extra-cellulaire minéralisée. La striation transversale périodique des fibrilles de collagène n'est pas totalement obscurcie par le dépôt minéral.

Mesoplodon densirostris is the bone that was found to have, so far, the highest amount of mineral [4,56]. The periotic and the tympanic bones of the dolphin *Delphinus delphis* show a mineral amount that is slightly lower [11].

All these bones are characterized by their extreme compactness and density and also by their rigidity and brittleness. However, the ways by which these bones reach an exceptional high mineralisation rate differ from those found in regular osseous tissues and they most probably lead to a structural organisation that differs from that observed in a regular bone (Fig. 2).

2.1. Rostrum of the toothed whale Mesoplodon densirostris

The organisation of the collagen component is observed in sections of demineralised specimens. Light-microscope examination of longitudinal semi-thin sections stained to reveal the collagen fibril arrangement shows secondary osteons (Figs. 3 and 4) oriented along the length of the rostrum (Fig. 4). Few narrow dark bands located between two secondary osteons are remnants of the primary bone. Semi-thin cross sections show large secondary osteons (300–500 µm in diameter) surrounded by smaller 'satellite' secondary osteons (Fig. 3) and the remnants of the primary bone

that has a higher collagen content but a lower mineralisation (Fig. 5). The sections show an abundance of osteocyte lacunae in both the primary bone and the secondary osteons (Fig. 3).

TEM microscopy of demineralised specimens shows collagen fibrils of approximately 100 nm in diameter with 67-nm periodicity (Fig. 6). In the secondary osteons, the main part of the rostrum bone, the fibrils whose diameter is around 20 nm form a very loose network (Figs. 7 and 8). In cross section, these thin fibrils appear to outline honeycomb-shaped spaces (Fig. 7) that are probably tubular and extend along the length of the rostrum, as shown in demineralised longitudinal section (Fig. 8). TEM observations of mineralised sections show that the mineral form closely packed rectangular units distributed in parallel ribbons (Fig. 9).

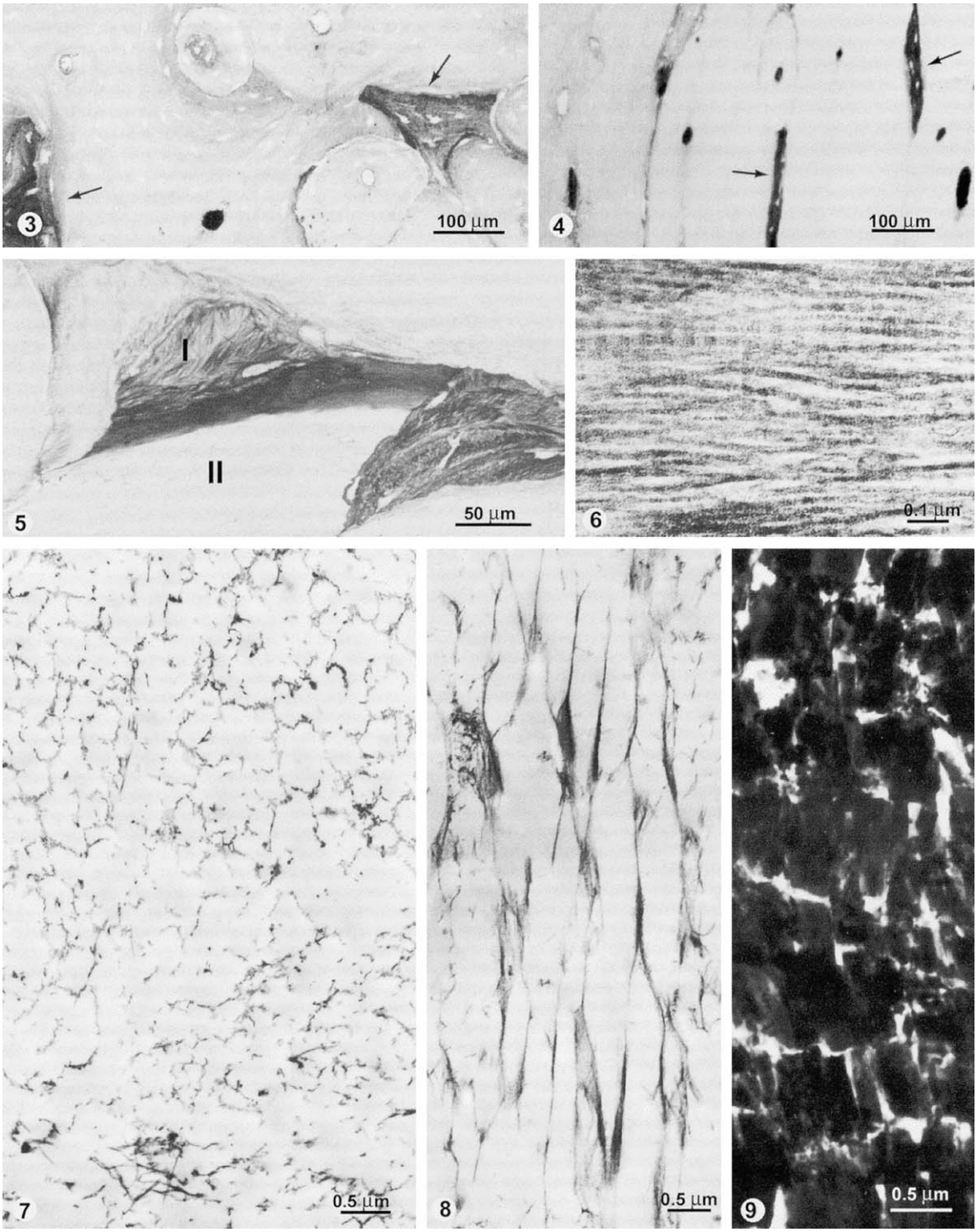
It is noteworthy that, in the rostrum, hypermineralisation is restricted to the haversian bone, but does not affect the primary bone that retains the characteristics of a 'typical' unspecialised osseous tissue. Unfortunately, the mineralisation processes that occur in the rostrum of *M. densirostris* have not been identified because the studies have been confined to fragments of a museum specimen.

2.2. Periotic and tympanic bones of Delphinus delphis (Table 1)

The periotic and tympanic bones (petro-tympanic complex) of the dolphin *Delphinus delphis* are also remarkable by their extreme compactness and density, and by their very high mineral content [44]. The histological context in which hypermineralisation occurs in the cetacean petro-tympanic complex is quite different from that prevailing in *M. densirostris* rostrum. Haversian substitution is not involved in this case; the lack of bone remodelling leads to an indefinite maintenance of the fibro-lamellar tissue, initially deposited as a loose spongiosa with hypermineralised trabeculae. In the dolphin, the spongiosa is already formed in foetus from 42 cm long. In this specimen, there are no inter-trabecular deposits that appear later in foetal growth. These deposits form perivascular bone: centripetal osseous layers centred on blood vessels (Fig. 10). The perivascular bone shows a weaker staining with toluidine blue than the trabecular bone. The basioccipital (for comparison with an unspecialised bone) is obviously more strongly stained than the petro-tympanic complex (Fig. 11). Before the end of the first year, the spongiosa is made compact by the development of the primary osteons that filled all the inter-trabecular spaces. The vascular network of bone, originally very extensive, is rapidly obliterated by the osteons. Ultrastructural studies carried out on a series of foetuses of different developmental stages and on adults revealed that the collagen fibrillogenesis is already modified in the earliest development stage examined. The collagen network is reduced by the rarefaction of the collagen fibrils and by the reduction of their diameter in both trabecular and perivascular bone. The collagen matrix of the trabecular bone is composed of thin collagen fibrils (about 30 nm in diameter) (Fig. 12). They are arranged in a dramatically reduced network compared to the basioccipital bone, where the closely packed collagen fibrils are of about 100 nm in diameter (Fig. 13). However, the 100-nm and the 30-nm collagen fibrils exhibit a similar banding pattern. The loose collagen network of the perivascular bone is composed of short collagen fibrils of about 15 nm in diameter (Fig. 14). In situ disassembly of the collagen fibrils obviously concerns the thicker fibrils and gives rise to very thin fibrils of 10 nm in diameter (Fig. 15). Conversely, the 15-nm fibrils reached their definitive diameter as soon as they arise from the osteoblast processes (Fig. 16). In the basioccipital, the diameter of the collagen fibrils is about 60 nm when the fibrils

Table 1
Mineralisation content in dense bones
Tableau 1. Contenu minéral dans l'os dense

Species	Bone	Mineral rate	Density (g cm^{-3})	Compacity
Mesoplodon densirostris	Rostrum	87%	2.6	99%
Delphinus delphis	Petrotic	84%	2.6	94%
Delphinus delphis	Tympanic	83%	2.6	98%
Delphinus delphis	Basioccipital	64%		
Bovine [12]	Femur	67%	2	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arise from the osteoblast processes and it increases in the extracellular space close to the osteoblast to reach its final size of 100 nm (Fig. 17).

It is noteworthy that the reduction of the collagen network by rarefaction of the fibrils and decrease of their diameter that occurs in *Osteogenesis imperfecta* (OI) leads to a decreased abnormal mineral deposit [7,8]. OI are inherited disorders characterised by brittleness of bone resulting from heterozygotous mutations of the genes coding for the type-I procollagen chains. Abnormal collagen molecules, incorporated during the fibrillogenesis are unable to form an organised network necessary to the deposition of the crystals [10].

The osteogenic process occurring in the petro-tympanic complex of the Cetacea obviously differs from the normal or pathologic mammalian situation. Whatever the age of the animals, there is no inner remodelling in the periotic and tympanic bones. Remodelling requires the destruction of the extracellular matrix that fails in the petro-tympanic complex. Rather than a failure of osteoclast competence, the lack of remodelling in the petro-tympanic complex could most probably be related to the early obliteration of the vascular canals that impedes the recruitment of osteoclasts.

The high mineralisation rates observed in both the rostrum of *Mesoplodon densirostris* and the petro-tympanic complex of the dolphin arise from quite different opposite histogenetical processes. In the petro-tympanic complex, the high level of the mineral deposit is reached through the maintenance of an embryonic woven-fibered bone due to the absence of Haversian remodelling. In contrast, in the *Mesoplodon* rostrum, there is a late (adult males) formation of Haversian tissue with hypermineralised secondary osteons. In spite of ontogenic differences, the *Mesoplodon* rostrum and the dolphin petro-tympanic complex show similar biochemical behaviour [13].

Functionally, the increase of the mineral content in these osseous tissues is supposed to increase their efficiency in the ultra-sound conduction [25]. Thus, the structural convergence of the *Mesoplodon* rostrum and the petro-tympanic complex suggests an acoustic role for the rostral densification [5].

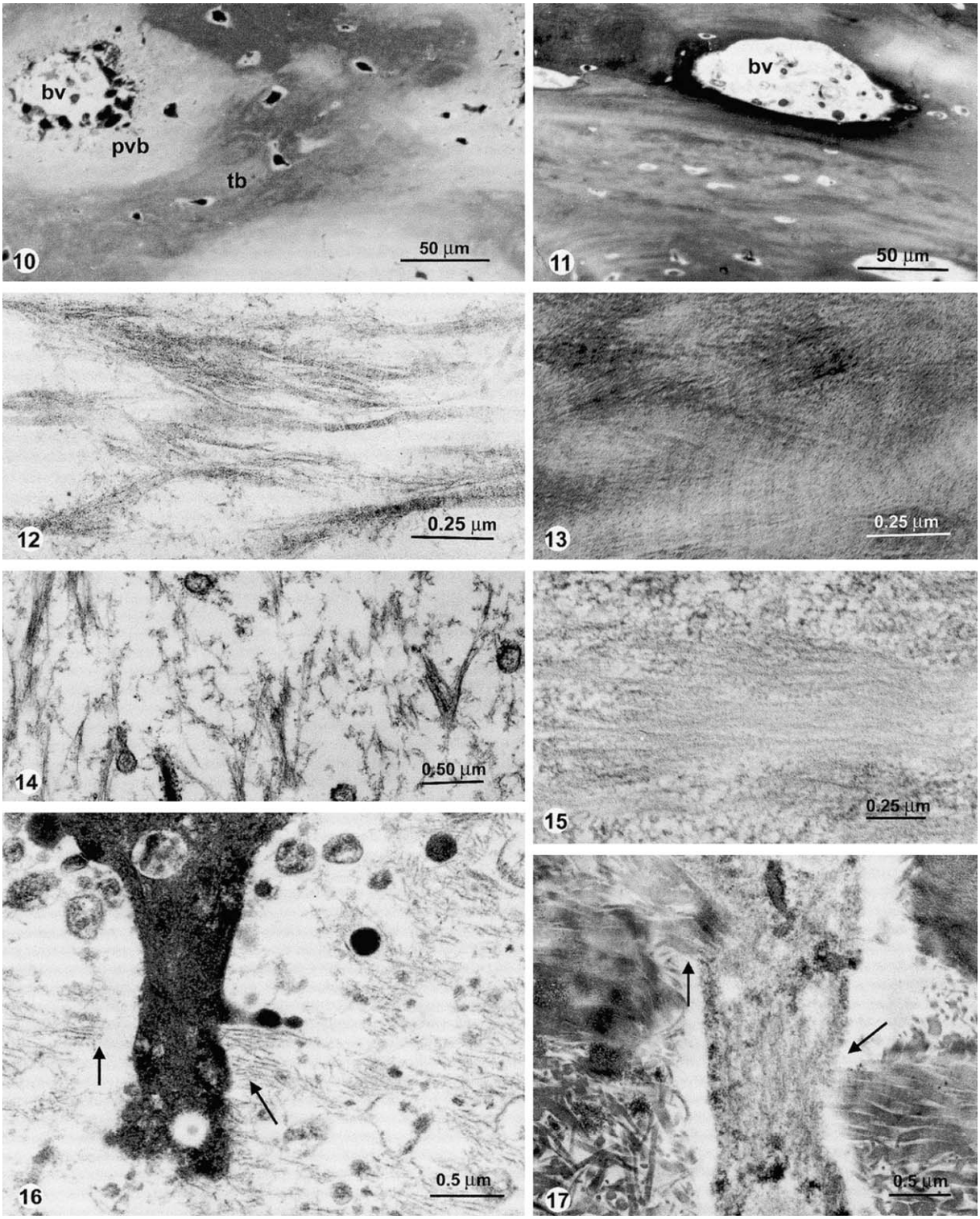
Contrary to these hard and brittle bones other bones are remarkable by their flexibility and a low mineral content that are the characteristics of the elasmoid scales of the teleost fishes.

3. Bones with a low mineral content: elasmoid scales in the teleosts (Osteichthyes)

In most living teleosts, elasmoid scales are thin, transparent, imbricated, lamellar, collagenous plates (definition in [1]) that are, at least in part, responsible for the swimming performance [6]. They are considered as dermal derivatives, because they mineralise without an intermediate, transitory cartilaginous stage [55]. Each scale set in a scale pocket surrounded by scale-pocket cells is lined by the scale-forming cells coined scleroblasts by Klaatsch [27]. The elasmoid scales are known to be composed of three layers differing in their histochemical, fine structure and mineralisation: the outer limiting layer, the external layer and

Figs. 3–9. *Mesoplodon densirostris*. Rostrum. **3**. Cross semi-thin section stained with toluidine blue, showing the longitudinal orientation of the secondary osteons. Arrows: primary bone remnants. **4**. Longitudinal semi-thin section stained with toluidine blue showing lightly stained secondary osteons and densely stained primary bone remnants (arrows). **5**. Detail of the primary bone remnants (I) where the organic matrix is denser than in the secondary osteons (II). **6**. TEM. Demineralised section of the primary bone remnant showing closely packed, banded collagen fibrils. **7**. TEM. Demineralised cross section of the thin collagen fibrils that appear as dots forming a hexagonal motif in secondary osteons. **8**. TEM. Demineralised longitudinal section showing the tubular aspect of the collagen network in secondary osteons. **9**. TEM. Mineralised section showing the closely packed rectangular mineral units.

Figs. 3–9. L'os hyperminéralisé : rostre de *Mesoplodon densirostris* (Cétacés). **3**. Coupe semi-fine transversale colorée au bleu de toluidine montrant l'orientation longitudinale des ostéones secondaires. Les flèches indiquent des restes de tissus osseux primaires. **4**. Coupe semi-fine longitudinale colorée au bleu de toluidine montrant les ostéones secondaires faiblement colorés et les restes fortement colorés de tissu osseux primaire (flèches). **5**. Détail des restes de tissu osseux primaire (I) où la matière organique est plus dense que dans les ostéones secondaires (II). **6**. Microscopie électronique à transmission. Coupe déminéralisée de restes d'os primaire montrant les fibrilles de collagène en phase. **7**. Microscopie électronique à transmission. Coupe transversale déminéralisée dans un ostéone secondaire ; les fines fibrilles de collagène forment un réseau hexagonal lâche. **8**. Microscopie à transmission. Coupe longitudinale déminéralisée dans un ostéone secondaire montrant l'aspect tubulaire du réseau collagénique. **9**. Même matériel, non déminéralisé, montrant les unités minérales de section quadrangulaire, juxtaposées.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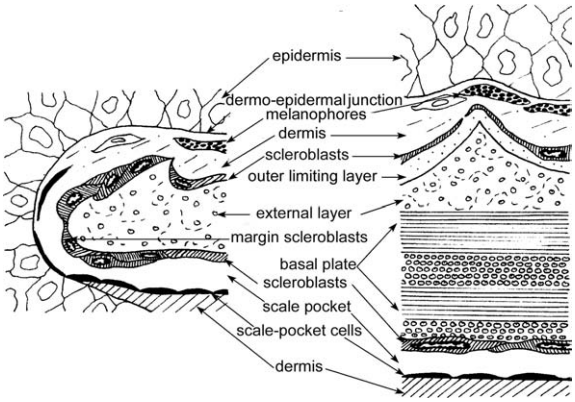
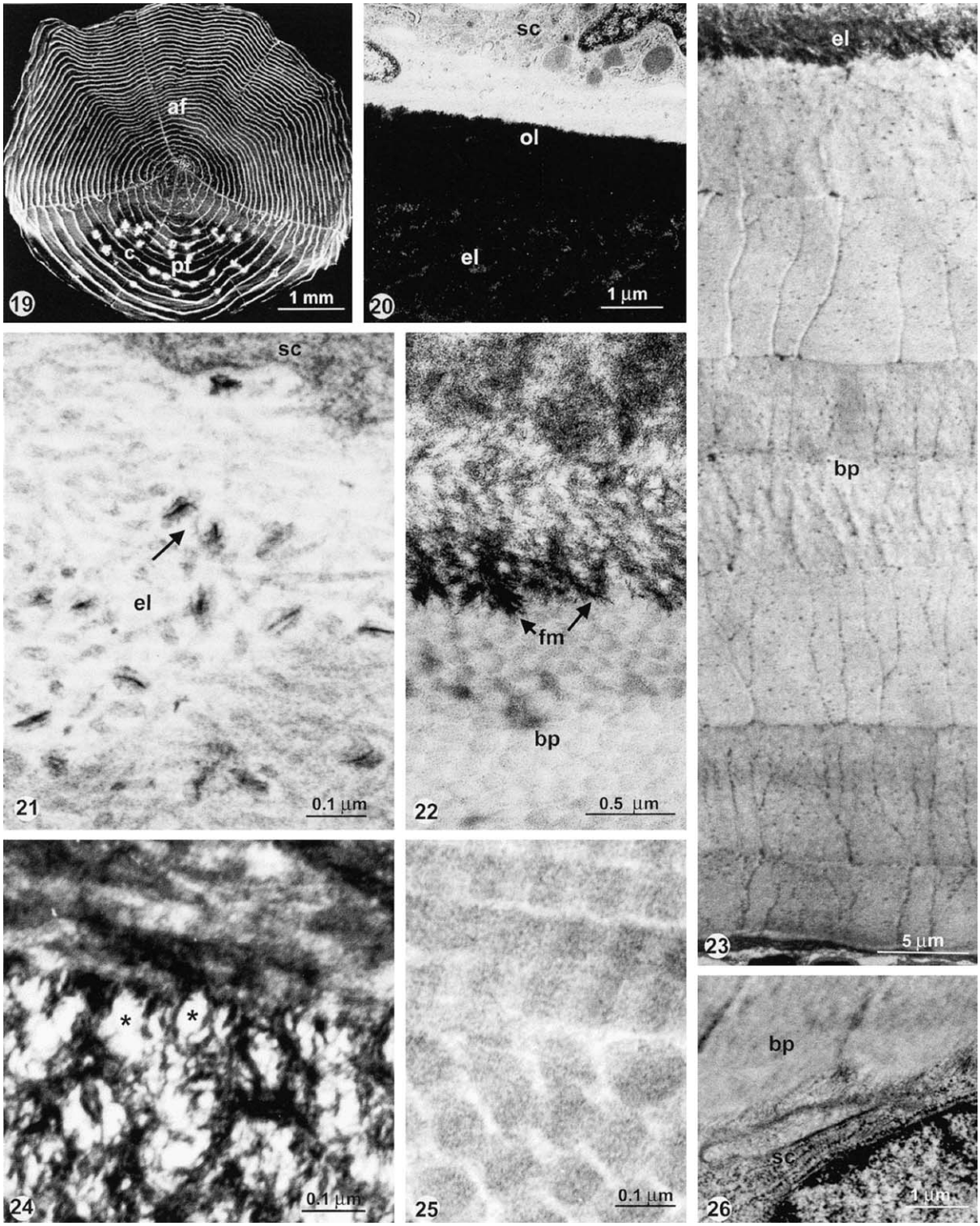


Fig. 18. Diagram of a section of a typical elasmoid scale. The section is perpendicular to skin surface.
Fig. 18. Écaille élasmoïde des Téléostéens. Schéma d'une section verticale d'une écaille élasmoïde typique. À gauche, en place dans la poche de l'écaille, en début de formation ; à droite, détail d'une écaille complètement formée, avec une épaisse plaque basale.

the basal plate [41] (Fig. 18). The outer limiting layer (OL) constitutes the outer surface of the scale in the posterior field of the scale (Fig. 19). In demineralised sections, the organic matrix appears as a delicate meshwork of thin microfibrils but does not contain collagen fibrils that are the main component of the two others layers. Biochemical analysis shows that type-I collagen is the predominant component and it is associated with the minor type-V collagen [54]. The external layer (EL) is located below the OL in the posterior field and at the surface in the anterior field. Elevations of the EL form concentric rings, the circuli, which ornament the scale surface (Fig. 19). This well-mineralised layer (Fig. 20) is made of thin collagen fibrils about 20–30 nm in diameter arranged in a loose network (Fig. 21). It mineralises first during ontogenesis. The mineralisation starts at the periphery of the scale (Fig. 21). Crystals do not appear to be associated to the thin collage fibrils. They seem to be rather related to dense granules distributed in the interfibrillary matrix [53]. Crystals are also observed within matrix vesicles [50]. The crystals aggregate to form clusters. The clusters increase in size by agglomeration of new crystals. Then, the clusters fuse and the EL becomes entirely mineralised (Figs. 20 and 23). The basal plate composed of isopedine [33] is the main part of the scale; it shows a lamellar structure. The thick collagen fibrils (about 100 nm in diameter) are arranged in superimposed ply. In each ply, the closely packed collagen fibrils are parallel to each other. Their direction varies from one ply to the next forming a plywood-like structure (Fig. 23). In some species like the goldfish (*Carassius auratus*) thin collagen fibrils of about 30 nm in diameter form sheets that are perpendicular to the plywood-like structure. These fibrils were called 'TC fibres' [35]. In the basal plate, the crystals are always oriented by the collagen fibrils and mineralisation is isotropic as in osseous tissues. The mineralisation of

Figs. 10–17. Petro-tympanic complex. **10**. Foetus 55 cm long. Semi-thin section in the periotic bone stained with toluidine blue. A more intense staining is observed in the trabecular bone (tb) than in the perivascular bone (pvb) surrounding the blood vessel (bv). **11**. Foetus 96 cm long. Semi-thin section of the basioccipital (considered as a control) strongly stained with toluidine blue. **12**. TEM. High magnification of collagen fibrils in the trabecular bone of the petro-tympanic complex of a 96-cm-long foetus showing the dissociation of the fibrils. **13**. TEM. Basioccipital of a 96-cm-long foetus. The closely packed collagen fibrils are organised in register (compare with Fig. 12). **14**. TEM. Petro-tympanic complex in an adult aged 13 years. Sparse thin collagen fibrils form a very loose network. **15**. TEM. Petro-tympanic complex of a 96-cm-long foetus. Disassembly of a collagen fibril. **16**. TEM. Foetus 55 cm long. In the petro-tympanic complex, thin collagen fibrils (arrows) arise from the osteoblast process. **17**. TEM. Foetus 55 cm long. In the basioccipital, the collagen fibrils arising from the osteoblast process are much thicker than in the petro-tympanic complex.
Figs. 10–17. L'os hyperminéralisé : le complexe pétrotympanique chez *Delphinus* (Cétacés). **10**. Fœtus de 55 cm de longueur. Coupe semi-fine dans l'os périotique coloré au bleu de toluidine. Une coloration plus intense s'observe dans l'os trabéculaire (tb) que dans l'os périvasculaire (pvb) entourant les vaisseaux sanguins (bv). **11**. Fœtus de 96 cm de longueur. Coupe semi-fine dans le basioccipital (pris comme contrôle) fortement coloré par le bleu de toluidine. **12**. Microscopie électronique à transmission. Vue à fort grossissement des fibrilles de collagène dans l'os trabéculaire du pétrotympanique d'un fœtus de 96 cm de longueur montrant la dissociation des fibrilles. **13**. Microscopie électronique à transmission. Basioccipital d'un fœtus de 96 cm de longueur. Les fibrilles de collagène accolées sont organisées en phase (comparer avec la Fig. 12). **14**. Microscopie électronique à transmission. Complexe pétrotympanique d'un adulte âgé de 13 ans. Les fibrilles de collagène peu nombreuses forment un réseau lâche. **15**. Microscopie électronique à transmission. Complexe pétrotympanique d'un fœtus de 96 cm de longueur. Dissociation des fibrilles de collagène. **16**. Microscopie électronique à transmission. Fœtus de 55 cm de longueur. Dans le complexe pétrotympanique, de fines fibrilles de collagène (flèches) émanent d'un processus ostéoblastique. **17**. Microscopie électronique à transmission. Fœtus de 55 cm de longueur. Dans le basioccipital, les fibrilles de collagène issus du processus ostéoblastique sont bien plus grosses que dans le complexe pétrotympanique.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fm
bp
0.5 µm
22
* *
0.1 µm
24
0.1 µm
25
bp
sc
1 µm
26

the basal plate starts after that of the EL, in the oldest plies of the basal plate adjacent to the EL. Then, the mineralisation progresses towards the inner part of the scale it starts at the periphery of the fibrils as in other mineralised tissues [45]. But, in scales, the mineral deposit remains mostly in the interfibrillary matrix (Fig. 22). The crystals do not penetrate deeply within the collagen fibrils that appear in cross-section as circular electron-lucent surfaces (Fig. 24) corresponding to the cross section of the collagen fibrils in the non-mineralised part of the basal plate (Fig. 25). The major axis of the crystals is parallel to the elongation of the collagen fibrils. The orientation and the location of the crystals are consistent with the data reported on the relationships between apatitic crystals and collagen fibrils, where type-I collagen is the major component [45]. In the basal plate, narrow interfibrillar spaces separate the very densely packed thick collagen fibrils, that could explain that the total amount of mineral in the scales is lower than that found in the bones of the same species [55] (Table 2).

Table 2
Mineralisation rate in the elasmoid scales. Mineralisation rate in the vertebra of *Tilapia rendalli*: 1.25 g cm^{-3}
Tableau 2. Taux de minéralisation dans les écailles élasmoïdes. Taux de minéralisation dans les vertèbres de *Tilapia rendalli* : 1.25 g cm^{-3}

Species	EL (g cm^{-3})	BP (g cm^{-3})
Rutilus rutilus	1.13	0.84
Eupomatus Gibbosus	1.31	0.97
Tilapia rendalli	1.25	0.89

Ultrastructural observations reveal that scleroblasts that could be considered as osteoblasts, since they synthesise a collagen matrix and ensure its mineralisation, originate from a papilla. Then, according to their location, they synthesize the thin collagen fibrils of the external layer if they migrate at the external surface of the scales or the thick collagen fibrils forming the isopedine if they line the deep surface of the scales. The gene expression of type-V collagen is proportionally higher in the scleroblasts distributed on the external surface than in scleroblasts lining the inner surface of the basal plate (Fig. 27). These results support the hypothesis that the diameter of the collagen fibrils could be regulated by the relative amount of type-V collagen interacting with the type-I collagen [31]. It is noteworthy that in the scales where TC fibres are present the basal scleroblasts synthesize the thin fibrils forming the TC fibres and the thick collagen arranged in the plywood-like structure (Fig. 26). Observations on the relationship between the extracellular collagen fibrils and intracellular cytoskeletal organisation strengthen the view that primary orientation of the

Figs. 19–26. **19**. SEM. Superficial surface of a typical elasmoid scale of the teleost: *Carassius auratus* (goldfish) showing the circuli (c), concentric elevations that are less numerous in the uncovered posterior field (pf) than in the covered anterior field (af). **20**. TEM. Scleroblasts (sc), scale-forming cells, line the superficial layer of the scale that is composed of two well-mineralised layers the outer limiting layer (ol) and the external layer (el). **21**. TEM. Early stage of mineralisation of the external layer (el). Crystallites are formed in matrix vesicles (arrow). They are not oriented by the collagen fibrils. **22**. TEM. Mineralisation front (fm) in the basal plate (bp). The collagen fibrils are surrounded by the crystallites that do not penetrate deeply within the fibrils. **23**. TEM. Partially demineralised section. The external layer remains mineralised, whereas the basal plate is completely demineralised. Note the thickness of the basal plate (bp) where superimposed strata form a plywood-like structure. **24**. TEM. Detail of the organisation of the crystallites in the basal plate of the goldfish. Crystallites are mostly located in the interfibrillary matrix. **25**. TEM. Detail of the organisation of the collagen fibrils in the non-mineralised basal plate. **26**. TEM. Detail of a basal scleroblast (sc) that produces the collagen matrix forming the basal plate (bp).
Figs. 19–26. L'écaille élasmoïde des Téléostéens. **19**. Microscopie à balayage. Surface superficielle d'une écaille typique du Téléostéen *Carassius auratus* montrant les *circuli* (c) des reliefs concentriques moins nombreux dans le champ postérieur recouvrant (pf) que dans le champ antérieur recouvert (af). **20**. Microscopie électronique à transmission. Les scléroblastes (sc) ou cellules formatrices de l'écaille jouxtent la surface superficielle de l'écaille constituée de deux couches bien minéralisées, la limitante externe (ol) et la couche externe (el). **21**. Microscopie électronique à transmission. Stade précoce de la minéralisation de la couche externe (el). Des cristallites se forment dans des vésicules de la matrice (flèche) sans être orientés par les fibrilles de collagène. **22**. Microscopie électronique à transmission. Front de minéralisation (fm) dans la couche basale (bp). Les fibrilles de collagène sont entourées par les cristallites qui ne pénètrent pas profondément dans les fibrilles. **23**. Microscopie électronique à transmission. Courbe partiellement déminéralisée. La couche externe (el) est minéralisée, alors que la plaque basale est complètement déminéralisée. On note l'épaisseur de la plaque basale (bp) où des couches superposées de fibres de collagène constituent un contreplaqué biologique. **24**. Microscopie électronique à transmission. Détail de l'organisation des cristallites dans la plaque basale de l'écaille chez le poisson rouge. Les cristallites sont principalement localisés dans la matrice interfibrillaire. **25**. Microscopie électronique à transmission. Détail de l'organisation des fibrilles collagéniques dans la portion non minéralisée de la plaque basale. **26**. Microscopie électronique à transmission. Détail d'un scléroblaste (sc) produisant la matrice collagénique sécrétant la plaque basale (bp).

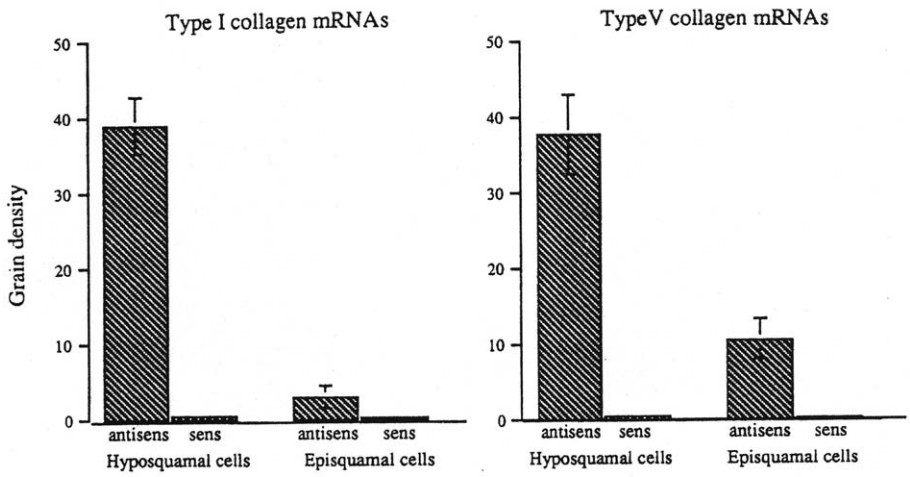

Fig. 27. In situ hybridisation. Quantification of the relative amounts of α1(1) and α(V) mRNAs in the superficial and basal scleroblasts of 6-day regenerating scale. The grain density was calculated as the ratio of grain areas evaluated by epipolarisation to cell areas measured from a bright field. (Modified from Le Guellec and Zylberberg, 1998).

Fig. 27. Hybridisation in situ. Quantification des valeurs relatives des mRNA pour les collagènes α1(1) et α(V) respectivement dans les scléroblastes superficiels et basaux de régénérats d'écailles de 6 jours. La densité des grains a été calculée comme le rapport des surfaces de grains à la surface des cellules évaluées par épipolarisation en fond clair. (D'après Le Guellec et Zylberberg, 1998).

newly formed collagen fibrils is controlled by the organisation of the cytoplasm [52].

4. Conclusions

From the diversity of bone tissues, it could be hypothesized that in each tissue osteoblasts may express only a subset of molecules from the potential osteoblast repertoire. The debate centres on how osteoblasts acquire positional information to express this subset.

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