Chapter 20

Microstructure and Mineralization of Vertebrate Skeletal Tissues


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I. Introduction

The parts of the vertebrate body which mineralize during life, comprising at least 60% of its dry weight, collectively form the skeleton. Besides its obvious mechanical function as the body's scaffolding, anchoring muscles and providing levers and articulations for locomotion, food acquisition and food processing, as well as holding and protecting fragile organs and soft tissues, the skeleton performs equally important (if less obvious) physiological functions during the life of the animal. Let us mention, first and foremost, its involvement in the storage and release of calcium and phosphorus salts in the control of body homeostasis. These varied and apparently conflicting functional demands are exemplified by the extraordinary process of skeletal growth, which accomodates simultaneous changes in skeletal size, shape and proportions with the continuous fulfillment of all its mechanical and physiological functions. Indeed, as a broken leg or a decayed tooth painfully reminds us, skeletal tissues are as much living parts of an organism as its skin, muscles, guts and nerves. On the other hand, because skeletons resist decay after death, they have become the awe-inspiring epitome of death itself in every human culture. In fact, a more ironic consideration of the vertebrate skeleton, as prompted by scientific comparative anatomy, encourages us to view it as a precise and informative 'summary' of the body's integrated level of evolution and adaptation to the environment.

It is noteworthy that, owing to their extensive mineralization, skeletal components are, with few exceptions, the only parts of the vertebrate body which become fossilized. Skeletons therefore permit us to study evolutionary changes throughout the geologic history of vertebrates. Indeed, in spite of the light shed on biological evolution by comparative anatomy, embryology, and now biochemistry and comparative molecular genetics of living animals, it should not be forgotten that vertebrate paleontology provides a tremendous amount of historical data, the evolutionary significance of which remains unique. Paleontology does not merely suggest hypotheses about how things could have happened, but instead documents what things did happen and when. Thus to a very large extent, interpretations of vertebrate evolution deal first and foremost with fossil skeletons.

But interpreting fossil skeletons depends to a large extent on a precise knowledge and understanding of skeletal structures and functions among living vertebrates. This introductory section therefore introduces to some of the varied perspectives and approaches through which an integrated perception of living vertebrate skeletal structures and functions may be reached. At the same time, this part sets the stage for later sections of this chapter.

The bold type face used for some terms in this chapter indicates that the word is presently referred to for the first time, and that a definition, if not provided in the same paragraph, is given later in the chapter (see Contents, above, and Subject Index, at the end of the chapter).

A. Vertebrate Hard Tissues: Definitions and Limits.

Although the concept of the vertebrate skeleton is first and foremost obviously linked with bone, it is clear that this is a gross oversimplification. In addition to bone, several mineralized hard tissues such as calcified cartilage, dentine, ganoine, enamel, cementum, etc. are components of the vertebrate skeleton. Conversely, some hard tissues are not mineralized and are not parts of the skeleton proper while some soft tissues, neither hard nor mineralized, are nevertheless, for reasons of function or homology, intimately associated with the skeleton, and may be parts of it.

On general grounds, considering the Protista and Metazoa as a whole, it is easier to define the skeleton by its most obvious function, mechanical support, than by its structure or composition. Very often, mechanical support is performed only by more or less rigid extracellular organic matrices, commonly polysaccharides or proteins, which sometimes undergo chemical changes resulting in sclerification or hardening of the tissue. Among skeletal proteins the collagen family is especially important, as well as keratins, while chitin is perhaps the most important skeletal derivative of complex glucides.

Hardness is commonly a consequence of the mineralization of an organic matrix in which various metallic ions (iron, magnesium, strontium, etc.) or silica may be involved. Among the various mineralization processes, calcification is probably the most common. In this case, the mineral salts are commonly calcium carbonates such as calcite and aragonite. Finally, ossification, peculiar to vertebrates, is the calcification of specific organic matrices made of collagen, non-collagenous proteins and complex glucides, by mineral deposits consisting primarily of poorly crystalline...
hydroxypatite, a calcium phosphate. Ossification is a complex phenomenon controlled by living cells of the mineralizing tissue and by the physical and chemical conditions prevailing at the level of the extracellular matrices undergoing mineralization. All hard tissues in the vertebrate skeleton are mineralized by apatites (although not all ossify) and most have a mesodermal origin (e.g., bone, cartilage, and dentine) irrespective of their location in the body and pattern of mineralization.

Derived from sclerotic mesenchyme of the embryonic mesoderm, the material of the endoskeleton differentiates deep within the body, below the striated musculature. It is first formed as cartilage, but is later associated with periosteal (perichondral) ossification and endochondral ossification. In its final, mature form the endoskeleton consists of bones of both periosteal and endochondral origin associated with permanent cartilages in the joints, and with tendons and ligaments which connect bones to muscles and to each other, respectively. The tendons and ligaments may themselves become mineralized and thus become integrated within the mineralized part of the skeletal system.

Derived in part from the embryonic dermatome mesenchyme, the dermal skeleton differentiates superficially in the body, externally to the striated musculature. It forms dermal (membrane) bone which differentiates directly into the skin dermis without an intermediate, transitory, cartilaginous stage. However, some cartilages (so-called secondary cartilages) are known to occur in small amounts in the dermal skeleton of birds and mammals (Hall, 1975).

The tooth tissues (and associated peridontal tissues, such as cementum, which is very closely related to bone) are also parts of the dermal skeleton. Their most important component tissue, dentine (of which the ivory of elephants, sperm-whales and a few other mammals is a specialized version) resembles bone compositionally but shows several structural and developmental peculiarities. Dentine shows a rich spectrum of structural variations (mesodentine, plicidentine, etc.). Some hypermineralized tissues of teeth (durodentine, mesodermal enamel, enameloids, etc.), while superficially close to enamel, are nevertheless specialized variations of dentine.

The origin of tooth enamel is quite distinct from the origin of other dermal tissues. 'True' enamel as defined for mammals has an epidermal origin and involves specific proteins (enamelin and amelogenin). Collagen is entirely lacking in its extracellular matrix.

It is important to recognize that, in spite of their specialized and restricted locations and functions in the mouths of 'higher' vertebrates, tooth tissues are ancestrally merely skeletal components of the vertebrate skin, differentiating through epidermal/dermal interactions and forming a dense, continuous covering over the body of primitive or generalized aquatic vertebrates. In the fishes, teeth more or less intimately fuse and integrate with other components of the dermal skeleton (flat bones, scales, fin rays, etc.). For example, ganoin, which occurs in the dermal skeleton of some fishes, appears to be a hypermineralized 'dental' tissue of epidermal origin, in all respects homologous with 'true' dental enamel of teeth (Sire et al., 1987). True epidermal enamel is known to occur in the teeth of vertebrates ranging from generalized osseous fishes to mammals.

Recent experimental data suggest that some components of the skull dermal skeleton may be derived from neural crest cells (Langille and Hall, 1988). This may also be true for the cells which form the scales (or parts of scales) in advanced bony fishes (Matsumoto et al., 1983). To the extent that some parts of the splanchnocranium (the mouth and anterior digestive duct in the head endoskeleton) are also known to have a neural crest origin, these new findings emphasize the amazing morphogenetic potentials of the neural crest cells. These cells may well comprise a much more important component of skeletogenic cells than previously recognized.

Because the term 'endoskeleton' refers to the deepest components of the skeleton, it is only natural that its antonym, 'exoskeleton', has been applied to the more external skeletal components, such as dermal bones, scales and teeth. However, from a general zoological perspective, the term 'exoskeleton' should be restricted to hard tissues which differentiate on the exterior of the epidermis. For example, the cuticles of nematode worms and the chitinous integument of arthropods are true exoskeletons. On the other hand, the superficial mineralized components of the vertebrate skeleton should be referred to as the 'dermoskeleton' because they differentiate within the skin dermis. One can include in the dermal skeleton enamel and enamel-like tissues which, irrespective of their epidermal origin, also differentiate close to the dermis and not outside the skin. From this point of view, the only true 'exoskeletal' structures in vertebrates are the hard, non-mineralized, keratinous derivatives which differentiate outside the skin epidermis, such as horns, claws, nails and the like. These keratinous exoskeletal elements are not presently considered parts of the 'true' skeleton and they will not be dealt with further here.

Finally, a peculiar situation is raised by some non-mineralized parts of fin rays and scales. It may appear paradoxical to accept such structures as parts of the skeleton, but indeed comparative and developmental studies (Meunier, 1987a) demonstrate their homology with mineralized skeletal elements. Considering that most or all parts of the endoskeleton in modern jawless fishes (lampreys, etc.) and chondrichthyns (sharks, skates and rays) consist of non-calcified cartilage, the apparent paradox of unmineralized tissues as integral parts of the vertebrate skeleton vanishes. A similar situation occurs among tetrapods where tendons and ligaments may or may not be ossified in closely related species.

B. Historical Overview of Bone and Vertebrate Skeletons.

Progress in understanding the vertebrate skeleton as an integrated and living part of the organism has been exceedingly slow and difficult. The renowned scientists of classical antiquity, such as Aristotle and Galen speculated on the nature of bones but apparently never reached a scientific assessment of the issue. Galileo (1638) recognized the relationship between bone shape and cross-sectional width and the mechanical function of weight bearing, thus initiating the concept of a link between bone structures and the mechanical demands imposed upon them. This concept met with success during the engineering-oriented second half of the nineteenth century, with the famous works of Culmann (1867) and Wolff (1892), among others, as summarized by Murray (1936) and Enlow (1963). This biomechanical perspective on bone structure still flourishes today.

Scientific experimental assessments of bone growth patterns and remodeling mechanisms were performed as early as the early Eighteenth Century (Belchier, 1736). But the renowned experiments of Duhamel du Monceau (1739, 1743), which involved feeding various mammals and birds
with madder and putting metal nails or rings in growing bones, remain a milestone in the field. These experiments were followed and refined by Hunter (1798), Flourens (1845) and Humphry (1856), among many others. Experiments of multiple labelling of growing bones in vivo with fluorochromes are modern analogs and extensions of these pioneering works.

A proper understanding of bone histology, or the fine microscopic organization of bone as a tissue, has puzzled scientists for centuries. As soon as early microscopes were developed, Leeuwenhoek (1674, 1693) and Havers (1691) pioneered microscopic descriptions of bone tissues. Progress in this field, in connection with the discovery and refinement of cell theory, was later made by Howship (1815), Müller (1836), Gegenbaur (1864) and Weidenreich (1930) among many others. More recently the electron microscope has opened an entirely new era in the fine description of bone tissue and associated cells.

Last but not least, the basic issue of the chemical nature of bone was successfully addressed as early as the eighteenth century, when Hérissant (1758), in famous experiments "using fire and acids", demonstrated the dual organic-mineral nature of bone.

It is noteworthy that the four major research fields mentioned above, namely, 1) relationships between structure and mechanical function, 2) growth and remodeling, 3) histological structure, and 4) the chemical nature of bone, are still with us today. Each of these approaches deals with a distinct level of integration of bone. This raises the point that, depending on the context, the term 'bone' can connote different concepts, such as an anatomical organ (a femur for instance), a tissue (the bone tissues which form every bone), or even chemicals (organic and mineral molecules characteristic of bone tissues). In order to clarify this issue, Petersen (1930) defined successive levels of integration of bone which, with some modification, have been generally accepted by later authors. These levels, listed in Table I, are used as the framework for the organization of this chapter.

C. Bone: Levels of Integration and the Present Chapter Organization (Table I).

It should be stressed that the following discussion of levels of integration, although using bone as the main example, applies equally well to the other components of the skeleton, such as articulations, teeth, etc.

First order structures. This is the anatomical level of integration in which the vertebrate skeleton appears, with the naked eye, as a system of several anatomical organs, the bones. This is the level of current descriptive and comparative anatomy, and also of classical vertebrate paleontology, as described in Section II, below.

Second order structures. This is the fine anatomical and tissue or histological levels of integration. Most studies at this level are performed with the optical compound microscope or SEM. The tissues include the bone itself, with all its variations, which have interesting functional significance, as well as cartilage and the soft tissues intimately associated with bone, such as periosteum, endostem, and tissues of ligaments and tendons. This level of integration, which pre-eminently deals with the problems of bone growth, shape and remodeling are dealt with in Sections III and IV.

Third order structures. Third order bone structures consist of cells, extracellular matrix and minerals which integrate to form the second order structures. They are preferably studied by TEM and SEM microscopy. Third order structures include the study of bone cells and other cellular categories, as well as the study of the extracellular matrices formed by these cells. This is the cytological level of integration of bone and is addressed here in Section V.

Fourth order structures. These structures deal with the molecular level of integration of bone components. Some of the issues addressed at this level include the specific organic molecules pre-eminent in bone matrix, such as collagen, proteoglycans and acid phosphoproteins, as well as bone minerals and the relationships between these organic and mineral components as realized by the biomineralization process. This is the finest level of integration of bone organization in modern biology, and it is studied by molecular, biochemical and various biophysical techniques (see Section V).

Note that the enumeration of the sections used in this chapter does not match, for practical reasons, that of Petersen’s classical four orders, as defined above.

Section VI of this chapter deals with non-osseous hard skeletal tissues. Apart from calcified cartilages and related tissues, this section includes dental and associated hard tissues of scales (among 'lower' vertebrates, especially). Other tissues which may mineralize, irrespective of pathological conditions, such as tendons and ligaments, are also covered in this section.

Sections II to VI offer definitions of the main terms used in the study of vertebrate hard tissues. Note that most definitions apply to structures, but some apply to processes or mechanisms. Whereas structures can be logically distributed under the various sections dealing with successive levels of integration, this is not the case for processes, which are essentially 'non-dimensional'. Thus the approach taken here is purely practical, with processes being defined where this appears to be logical or appropriate for various reasons.

Some definitions are accompanied by proposed equivalents in French or German, but no attempt has been made to offer such translations systematically. Synonymies or translations are offered only when they refer to French or German expressions which are not the direct counterparts of the English terms, when they have some special historical interest in earlier classical literature, or when they are linked to the historical emergence or evolution of concepts. French and German translations are omitted when they would be obvious or straightforward.

Finally, Section VII offers concluding remarks on the comparative structures of vertebrate skeletons viewed in an evolutionary perspective.

D. The Anatomical Organization of the Vertebrate Skeleton: First Order Structures.

Only the general anatomical organization of the vertebrate skeleton is reviewed here to provide an introduction for the more detailed surveys at other structural levels (Figure 1A).

The anatomical organization of the vertebrate skeleton is based on the general location of the various elements and their mode of development. One can first distinguish the axial skeleton from the zonal (girdle) and appendicular one. Each can contain paired and non-paired bones, either of dermal or endoskeletal origin. The axial skeleton consists of the cranium (or skull) and the postcranial axial skeleton. The cranium itself combines two main components: a dorsal one, the neurocranium, which associates with the brain and
TABLE 1. Successive levels of integration of bone; modified from Petersen (1930).

<table>
<thead>
<tr>
<th>Order of Structure</th>
<th>Approximate Scale</th>
<th>Structures Characteristic of the Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANATOMICAL LEVEL OF INTEGRATION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIRST ORDER</td>
<td>1 mm - 1 mm</td>
<td>Bone and tooth morphology, number and relationships; vascular orientation in compact bone; trabeculae in spongy bone.</td>
</tr>
<tr>
<td>SECOND ORDER</td>
<td>1 mm - 100 μm</td>
<td>Orientation, size and number of bone trabeculae; size and number of vascular canals; structure of extracellular matrices.</td>
</tr>
<tr>
<td>CYTOLOGICAL LEVEL OF INTEGRATION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>THIRD ORDER</td>
<td>100 μm - 1 μm</td>
<td>Details of cells; details of extracellular matrices; orientation, amount, organization, relationships.</td>
</tr>
<tr>
<td>MOLECULAR LEVEL OF INTEGRATION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOURTH ORDER</td>
<td>1 μm - 10 μm</td>
<td>Chemical and biophysical organization of organic and mineral components.</td>
</tr>
</tbody>
</table>

sensory organs of the head, and a ventral one, the splanchnocranium, which forms around the mouth and anterior digestive duct. Both the neurocranion and splanchnocranium contain skeletal elements of dermal and of endochondral origin. The endoskeletal elements differentiate deeper in the head, first as cartilages, comprising the chondrocranion or cartilaginous skull. Although present only in a transitory stage in the embryo of most vertebrates, the chondrocranion forms most of the skull in adult sharks. The dermal components of the neurocranion differentiate directly within the head dermis, forming the flat bones of the skull roof, snout, cheeks and roof of the mouth. Many of these bones bear teeth (vomer, ectopterygoid, dermopalatine, etc.), and are thus called the dentigerous bones.

The splanchnocranium differentiates as paired arches alternating with the paired lateral pharyngeal openings, which form the branchial slits. Each arch belongs to the endoskeleton and has a cartilaginous origin, but each may also be associated with more superficial bony components of dermal origin. As it is well known, the first two pairs of arches experience extensive changes in the jaw-bearing vertebrae (or Gnathostomata). The first pair becomes the mandibular arch differentiating into jaws (upper and lower) associated with dermal dentigerous components (premaxillary, maxillary for the upper jaw, dentary, etc. for the lower jaw). The paired slits placed before this mandibular arch may fuse together and form the mouth opening itself. The second pair of arches will form the hyoid arch which may bear several dermal bones forming the opercular system. It often functions, among fishes, to link the jaw apparatus and splanchnocranium as a whole to the neurocranium. The remaining pairs of arches become the branchial arches, often associated with small, dentigerous dermal bony plates.

The postcranial axial skeleton consists mainly of the unpaired endoskeletal bones, the vertebrae, which vary greatly among vertebrates in shape, structure and number. With them are associated the ribs, paired endoskeletal structures which are more or less spread along the vertebral column according to the vertebrate group. With the vertebrae and (or) ribs are associated several other bones (sternum, sternebrae, ventral ribs, chevron bones, urophore complex, etc.) the development of which varies tremendously from group to group.

The non-axial skeleton can be divided into a girdle (or zonal) skeleton, which forms the supporting girdles of the paired fins or limbs, and an appendicular skeleton which forms the unpaired fins and the paired fins in fishes, as well as the limbs in tetrapods (or terrestrial vertebrates).

The girdle skeleton forms anteriorly a thoracic (or pectoral or scapular) girdle, composed of endoskeletal bones (scapula, coracoid) on which the limbs bones always articulate, and superficial dermal bones (clavicle, cleithrum, etc.) which, among bony fishes, are connected to the dermal skull roof. Among tetrapods, an unpaired ventral dermal bone (interclavicle) unites left and right pectoral girdles while the dorsal connections with the skull roof are lost.

The pelvic girdle is endoskeletal and articulates with the rear paired (pelvic) fins or limbs. Free from the vertebral column among fishes, it is connected to it among tetrapods owing to one or several pairs of specialized sacral ribs.

The appendicular skeleton differentiates into the paired fins (of fishes) or limbs (of tetrapods). The fin skeleton is known as the pterygium, and the limb skeleton as the
<table>
<thead>
<tr>
<th>Examples of Techniques Used</th>
<th>Biological Problems Related to the Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissection, injection of vascular system, surgical experiments, X-ray, sawing observation: naked eye, binocular microscopy.</td>
<td>Comparative anatomy and morphology of the skeleton; overall growth; systematics and phylogeny; biomechanical adaptation to strains.</td>
</tr>
<tr>
<td>Cell and tissue culture observation: higher powers of optical microscopy, polarizing microscopy, SEM, TEM.</td>
<td>Cytology, cytochemistry of bone and dental tissue cells; expression of phosphocalcic metabolism at cellular levels, ultrastructural studies of matrices.</td>
</tr>
<tr>
<td>TEM, X-ray diffraction microprobe; biochemical and chemical techniques; molecular biology.</td>
<td>Ultrastructural relationships between cell (de)mineralization organals and extra-cellular matrices; biomineralization processes, study of biominerals, biochemistry and chemistry of organic and mineral components and their relationships.</td>
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chiridium. The pterygium consists of several inner endoskeletal elements which vary enormously in number and shape among fishes, and of dermal rays (lepidotrichia, etc.). The chiridium comprises endoskeletal elements only. The unpaired fins of fishes contain both endoskeletal and dermal bones as do the paired fins. The scales of fishes form a more or less continuous dermal skeleton on the body, which modulates locally into the specialized dermal elements of fins (fin rays or dermotrichia sensu lato) on one hand, and of the mouth and pharynx (teeth) on the other. Among tetrapods, various dermal derivatives devoid of dental tissues can mineralize and form scales, osteoderms, osteocutes or even ‘armor plates’ of variable fine structure, and more or less close to typical bone.

E. Skeletal Growth Constraints and Opportunities

In order to place the microstructural peculiarities of the vertebrate skeleton into a proper perspective, some out-group comparisons are necessary, especially as far as skeletal growth is concerned.

The fine processes of skeletal growth are exceedingly diverse if one takes into account the various mineralized skeletons of protozoans and metazoans. In most cases, however, a common constraint on skeletal growth is that it is non-intussusceptive, i.e., it does not involve hard, mineralized materials expanding from within. Growth must proceed by deposition of new hard material on the free (often external) surfaces of the skeleton (appositional or accretional growth). Indeed, the skeletal shapes and structures and the biological peculiarities of their component tissues are not independent of the growth processes involved. In fact, not only do the growth processes themselves control the very nature of the skeletons and their possible forms, but they also severely constrain their evolutionary patterns. Thus, skeletal structures cannot be understood in terms of adaptations to adult biological functions such as mechanical support, mineral reservoir, protection, etc., without consideration of the topological and geometrical laws which constrain their growth.

Among the more ‘primitive’ stages of skeletal evolution, accretional processes of deposition are the only ones involved in growth, and resorptive processes are not necessary. But, of course, the accretion rate is often cyclical and may sometimes fall to zero, either locally or in the whole skeleton, thereby accounting for ‘growth lines’ sensu lato. Such accretional processes, whether continuous or cyclical, impose more or less stringent limitations, for topological reasons, upon the kind of skeletal shapes which can evolve. Hence, skeletal growth under such circumstances often involves simple proportional relationships, growth along a generating spiral, growth in one or several planes, or growth along dichotomous patterns, and various combinations or alterations of these patterns.

Despite these inherent limitations, accretionary growth permits the evolution of skeletal architectures with complex forms and often with magnificent regularity. Such are the conditions of growth of spicules, tests and skeletons of various protozoans, sponges, corals, brachiopods, molluscs, and so on. In most cases, the skeleton is either entirely devoid of articulations, or it consists of a small number of component parts, which are not jointed or are very simply articulated.

In more advanced stages of skeletal evolution, one can find many varied articulations (mobile joints) between the parts of the skeleton, which function in complex ways in three
Fig. 1. Bones as skeletons and organs. A: The complete skeleton of a vertebrate with component bones kept in anatomical connections shows the main anatomical regions viz. the axial skeleton (skull and vertebral column), zonal skeleton (stippled) and appendicular skeleton. B: The three main morphological bone categories of the vertebrate skeleton are (1) long bones, (2) short bones, and (3) flat bones. C: Microanatomical details of three morphological categories; C1 = long bone with distinct fine anatomical organizations in (a) epiphyseal, (b) metaphyseal, and (c) diaphyseal regions. C2 = Flat bones have a three-layered structure known as diploe, with two thin cortical compacta separated by an inner cancellous region. C3 = Short bones commonly have mainly cancellous structure with a thin cortical compacta. Sources: A and B from Lessertisseur and Saban (1967); C from Lessertisseur and Saban (1967) and Ricqlès (1975).

dimensional space. This allows the skeleton to fulfill the various functions of protection, mechanical support and predation, among others, while still affording a great mobility.

However, the growth of such complex skeletal forms cannot generally be accomplished by the simple homothetical extension of its component parts, by external accretion only. Indeed, if this were so, the weight and thickness of the various elements would rapidly reach undesirable values as size increases.

For a given building material, maintenance of structural adaptation with increasing size generally requires extensive changes in the relative proportions of the structure (Thomson d’Arcy, 1917; Gould, 1970). Such changes in hard skeletons cannot always be accomplished by simple accretional deposition. For instance, the ratios of curvatures of articular
surfaces relative to the linear growth of the articulated components of the skeleton would have to change when overall size increases, and this is hardly accommodated by accretion only. For such reasons, further growth of complex articulated skeletons will need not only positive or null accretion rates, but indeed accretions with negative rates, or resorption. This apparently paradoxical situation is especially obvious if one considers growth of bones as organs (e.g., Enlow, 1963) (Figure 6B).

This growth-linked resorption is represented by very different processes as among different zoological groups. Among arthropods, the challenge raised by growth of a hard external skeleton with many articulations is solved by molting: growth resorption is complete and cyclical. The hard skeleton is temporarily eliminated and then replaced by a larger one, the process often involving changes in the proportions of the various parts (allometries). This solution is of course possible for true exoskeletons only, but it constrains arthropods, for biomechanical reasons, to very modest sizes, at least out of the aquatic environment.

Among vertebrates, skeletal growth resorption is incomplete and continuous. The skeleton is constantly experiencing localized resorption, which is quite obvious in the histological structure of bone tissue. This bone remodeling is growth-linked. The interplay of various hormones affords a fairly well understood systemic control of the equilibrium between bone deposition and resorption as a whole, but many factors, which control the fine, local, activity of cells, remain rather poorly known.

The vertebrate solution of growth resorption is well adapted to an endoskeleton and, in contrast to the arthropodan answer, it does not involve a critical growth phase comparable to molting. On the contrary, it reconciles structural change with continuous activity, fine mechanical adaptation with actual biomechanical demands, and imposes few constraints on reaching very large sizes in the course of evolution. The vertebrate answer also offers a possible reservoir of calcium and phosphorus ions for the other systems of the organism, according to its physiological demands.

It is thus obvious that the biology of skeletal growth has far-reaching consequences for every aspect of the relationship between structure and function among metazoans. Skeletons are not just epiphenumena grafted upon the basic bauplans of organisms but deeply rooted integral parts of these bauplans themselves.

F. Fossilization of Vertebrate Skeletal Tissues

Fossilization processes of vertebrate skeletons are varied and much has still to be learned in this field, although many works have been recently devoted to the paleoecological, taphonomic and geochemical aspects of the problem (e.g., Behrensmeyer and Hill, 1980; Hare et al., 1980; Shipman, 1981). Under circumstances leading to fossilization, a buried vertebrate skeleton will generally face physical and geochemical conditions which will induce more or less extensive and drastic changes to both organic and mineral components, at all levels of integration.

First order structures. Anatomical arrangement may suffer relatively few changes, and hence the external size and shape of bones may be directly studied according to the classical methods of comparative anatomy. However, compressive or shearing stresses most often produce post mortem cracks, crushing and sometimes extensive plastic deformation, all more or less greatly altering original shapes. The natural cavities of bones, such as medullary cavities or sinuses, are often filled by sediments or minerals (Rogers, 1924). Very small cavities within bone tissue such as thin vascular canals or cell lacunae and canaliculi are often infilled by iron or manganese oxides. Such oxides commonly also infill the minute inframicroscopical spaces of what is left of bone fine texture, giving fossil bones their peculiar brown, pink, yellowish, grey or dark colors. Substitution of the original bone mineral by sediment can sometimes take place in such a way that the fossil bone, although keeping its original form, is merely a natural cast of the original bone. In such circumstances, of course, fine structures are entirely lost and microscopical study of the fossil brings no additional information. Although it is still generally believed that this circumstance prevails in most cases of vertebrate fossilization, complete disappearance of fine structures is fortunately the exception rather than the rule among vertebrate fossils. In most circumstances, fine anatomical, histological, cytological, and even biochemical and biophysical features are to some extent preserved. Hence paleoecology and paleobiocchemistry are active fields of investigation.

Second order structures. Disregarding exceptional circumstances of fossilization, little of the organic phase of bone is left for study at the fine anatomical and tissue levels. The paleohistological studies which are nevertheless possible at this level can best be understood by reviewing the fate of the mineral phase of skeletal tissues during fossilization. As fossilization takes place, the mineral component of bone and other vertebrate hard tissues, namely minute crystals of hydroxyapatite, may stay basically unchanged for considerable periods of time. The arrangement of the tiny crystals, especially, may not change significantly as the porosity of the skeletal material becomes filled by exogenous minerals. This explains why fine anatomy and even histology can remain essentially unchanged in fossils and can be readily studied in most cases.

In most skeletal tissues the spatial organization of the minute apatite crystals is induced by the fine spatial arrangement of the organic phase, especially the collagen fibrils, in the process of inotrop mineralization. This spatial organization is mostly retained in fossilized hard tissues, even after the organic matrix has more or less disappeared. Using polarized light, it is possible to decipher the fine spatial organization of the apatite crystals and hence of the original organic matrix, even if the latter has mostly disappeared.

In most cases, however, the minute hydroxyapatite crystals are not entirely unchanged during fossilization. Because they are so small, they offer a tremendous external surface relative to their volume, forming an efficient trapping system for various ions introduced by percolating waters, such as fluoride (Parker et al., 1973). Chemical analyses of fossil bones generally show that they are richer in fluoride than modern bone. However it is still unknown whether the fluoride merely adsorbs onto the surface of apatite crystals, or if it substitutes for hydroxyl radicals within the lattice of apatite crystals themselves, to form true fluorapatites. The trapping properties of fossil bone for various heavy metals, especially uranium, is noteworthy. Autoradiographs of thin sections from uranium-rich fossils show, after activation in an atomic reactor, that the uranium atoms are trapped within
the apatite trabeculae of the fossil bone rather that in the infilling matrix. Simultaneous addition or substitution by calcium carbonate in fossil bone is also a current problem in vertebrate paleontology.

In most cases, fossil bone mineral is a calcium-rich apatite with various ions, including fluorine, superficially adsorbed and, at least to some extent, substituted into fluorapatite. Because of these and other diagenetic processes, fossil bones can vary considerably in their mineralogical structure and composition (Paine, 1937). For instance, the size of fluorapatite crystals in fossil bone is known to be rather diverse, but it should generally increase according to the absolute age of the fossil (Mosebach, 1974). However, 'young' fossils are sometimes known to have larger-grained apatite crystals than much older ones.

Third order structures. Direct studies of fossilized cells are not possible except in highly exceptional circumstances, such as mammoths frozen in ice, Eocene mitoses in epithelial cells originally fixed in acidic fossil peat bogs, and the like. However, size, shape of cells and their relative abundance per unit volume of tissue can be ascertained precisely in fossil bone, owing to the fact that cell spaces are often naturally 'cast' as preserved above and because the glucid-rich cell lacunae are commonly well preserved (Neves and Halstead-Tarlo, 1965; Halstead-Tarlo and Mercer, 1966; Pawlicki, 1975, 1984). Although these possibilities have seldom been pursued for fossil vertebrates (e.g., Vialli and Vialli, 1969; Thomson, 1972; Brambilla, 1972), they could bring, within selected lineages, some interesting data on problems of cell size and perhaps evolution of nucleo-cytoplasmic ratios.

As far as extracellular matrices are concerned, some TEM studies have been conducted on fossilized vertebrate tissues. While some techniques at best reveal the 'ghost imprint' of collagenous fibrils on the fossil minerals (e.g., Pawlicki et al., 1966), some studies have shown with TEM that even after demineralization of fossil bone and dentine, some collagenous fibrils (and many bacteria of post mortem invasion) were still preserved. Surprisingly, so far, there seems to be little relationship between the quality or amount of collagen fibril preservation, as observed by TEM on demineralized fossil materials, and the amount of proteins preserved, as observed by biochemical analyses of the same fossil material. This may reflect differential preservation of non-collagenous acid phosphoproteins relative to the collagen itself.

Fourth order structures. Following burial, the organic phase of bone suffers more or less rapid and complete decay. The prevalent organic component, collagen, is for the most part hydrolyzed into its amino acids components. Although free amino acids are often found in fossils, it is not always possible to tell whether they came from the fossil or from the neighbouring water-saturated sediments. Some amino acids may also come from the decay of bacteria and/or fungi associated with the decaying carcass. However, recent studies have shown that amino acids diagnostic of collagen, such as hydroxyproline, are indeed found in some fossil bones (Cohen-Solal et al., 1987). This provides strong circumstantial evidence that at least these amino acids came from the organic fabric of the bone. It is now also known that most amino acids contained in fossil bones are not free, but remain linked together by peptide links in the form of rather light peptides from at least 2000 to more than 3500 daltons in molecular weight (Cohen-Solal et al., 1987, and unpublished data). This may not be sufficient for studies of biochemical taxonomy. However, if characteristic epitope configurations are retained in some cases, this may provide the basis for meaningful 'paleoimmunological assays', where relative immunological distances between selected living and fossil specimens could be directly tested (Lowenstein, 1985).

Recent studies of amino acid composition of fossil bone and dentine suggest that the amount of organic components preserved is very small: from 0.010 to 0.016% in dry weight of fossil bone, on the average, compared to circa 30% in modern dry bone (Cohen-Solal et al., 1987). These studies also suggest that fossilization results in inversion of the relative amounts of collagenous versus non-collagenous 'proteins' preserved. While the initially very abundant collagen seems to be mostly hydrolyzed, a significant percentage of the initially rare non-collagenous bone proteins, notably the acid phosphoproteins, are commonly preserved, sometimes without much biochemical change (Ulrich et al., 1987). This may reflect their intimate link with the mineral phase of bone (Glimcher, 1984).

Although the initial protein content of mature enamel is very low (about 2% or less dry weight for mature enamel), the enamel proteins seem, on the average, to sustain a far better rate of preservation among fossils than those of bone and dentine, perhaps because of the 'closed' environment formed by the non-porous enamel.

In addition to proteins, some lipids (Pawlicki, 1976) as well as glucidic structures are left in vertebrate fossils (Isaacs et al., 1963; Halstead-Tarlo and Mercer, 1966; Sacchi-Vialli, 1967; Everts et al., 1968; Pawlicki, 1977). This evidence justifies the interest in 'paleochemical' investigations which began 30 years ago and which are active today (e.g., Runnegar, 1986).

In fossil skeletal material, all possible transitions exist between 'nearly perfect' histological preservation, sometimes even enhanced by natural 'staining' by metallic ions, and situations in which all the original mineral components have become recrystallized and/or replaced by other minerals. For instance, localized substitutions by pyrite or calcium carbonate crystals are common. Partial recrystallization and substitution may destroy fine histological details, but it may leave the gross microanatomical fabric generally undisturbed. Replacement of vertebrate hard tissues by silica is exceedingly rare, although this is quite common for fossil plants. Finally, post-mortem artifacts produced by fungal or bacterial invasions, post depositional cracking and other high pressure deformation all more or less enhanced by exogenous invasions of various minerals, notably iron, should be taken into account for proper interpretation of fossilized histological structures. Thorough experience gained from extensive comparisons with hard tissues of living vertebrates is exceedingly helpful in this regard.

II. The Morphological-Anatomical Level: Bones as Skeletons and Organs

In this section, skeletal elements are viewed as anatomical organs which comprise the entire skeleton. Apart from size, shape, number and relative location in the body, many textural details are obvious using the naked eye, gross radiography, a magnifying glass or the binocular microscope. Simple preparations, such as gross sawing, can supplement considerably the amount and quality of information available
at this level. The nature and texture of the skeletal tissues can in many cases be readily recognized at this level.

A. General Categories of Bones

1. Endoskeleton (French: squelette interne, squelette profond; German: Innerskelet). Literally 'the skeleton from within', this term refers to the internal position in the body of the skeletal elements involved, and to their ontogenetic development, i.e. pre-formed by a cartilaginous matrix, hence the name 'substitution bones' (French: os de remplacement, German: Ersatzknochen). However, not all endoskeletal material is formed by the substitution of bone to cartilage, since a great part is formed by periosteal or endosteal deposition (see below). Hence the concept of 'substitution bone', is not entirely valid for all endoskeletal elements, and, strictly speaking, it refers only to the endochondral component of endoskeletal bones. Moreover, this concept is commonly of little value, at least among 'higher' terrestrial vertebrates such as adult mammals, for deciphering relevant characteristics of endoskeletal bones. Indeed, the endochondral origin of some of the component tissues in a given bone in the adult endoskeleton is not necessarily obvious. However, all the well-formed, mobile joints involving functional articular cartilages are generally formed by endoskeletal elements, which mostly integrate endochondral ossification (see Section III). Notable exceptions are the mammalian lower jaw (dentary bone), a dermal bone which nevertheless bears a typical functional joint. Conversely, the vertebrae of some advanced bony fishes (teleosts) are typical endoskeletal bones which sometimes involve little, if any, endochondral ossification (François, 1966, 1967). However, among living lower vertebrates, endoskeletal bones may retain extensive cartilaginous compositions, irrespective of their articular surfaces.

Compact bone forming the long bones shafts is most commonly derived from periosteal ossification (see section III). Spongy bone forming epiphyseal and metaphyseal regions, as well as the endosteal margin, are formed by endochondral ossification and endosteal bone deposition (see section III). Apart from cartilaginous articular surfaces, encapsulated under ligamentous articular pockets, the endoskeletal bones are covered by a periosteum, a fibrous membrane associated with the insertions of tendons and ligaments. These bone surfaces are never 'sculptured' or 'ornamented', unlike dermal bones.

2. Exoskeleton (integumentary skeleton pro parte; French: squelette tégumentaire). Literally, 'the skeleton from without', the exoskeleton includes skeletal elements which differentiate from the external surface of the skin epidermis. Among vertebrates, the corresponding tissues are characterized by a high content of keratins, as in horns, nails and claws. With some exceptions (Moss, 1969b), most authors exclude these non-mineralized structures from the vertebrate skeleton (sensu stricto). The current use of 'exoskeleton' as an antonym for endoskeleton to refer to the superficial elements of mineralized vertebrate skeletons is inappropriate and should be avoided.

3. Dermoskeleton (dermal skeleton; French: squelette dermique). The dermoskeleton includes bones originally formed in the skin dermis, the teeth, tooth-like organs and tissues or their derivatives among the 'lower' vertebrates, and the bony scutes of terrestrial vertebrates.

Dermal bones are commonly (but not always) flat and they lack typical mobile joints. They form in the dermis via direct (membranous) ossification, without differentiation of transitory cartilages. However, minor cartilages ('secondary cartilages') associated with the dermoskeleton are known to differentiate in small amounts in embryos of mammals and birds (Hall, 1978). They commonly form under transitory, pathological or experimental circumstances, and peculiar localized biomechanical conditions are generally involved in such cases.

Dermal bones may become secondarily associated with endoskeletal elements, fusing with them to form composite or mixed bones such as the 'angular' bone of the teleost fishes lower jaw, the 'supra-occipital' bone in many mammals, and the 'temporal' bone in human anatomy.

Among 'lower' aquatic vertebrates, amphibians and reptiles, dermal bones may show superficial 'ornamentation', such as pits, grooves, tubercles, ridges, etc., commonly organized in patterns indicative of the overall growth trend of the individual bone (Bystrov, 1935; Buffrénil, 1982). Also, the ornamentation of several adjacent but anatomically distinct bones may become integrated to form general patterns linked to local or overall growth trends, e.g. on the skull roof or the lower jaw (ibid.).

In non-tetrapod vertebrates, dermal bones are commonly intimately associated with superficial dermal tissues and the superficial part of dermal scales. The dermal components may be organized as superficial isolated simple dermal units (odontodes), or they may fuse together, following different patterns in various lineages, to form 'odontocomplexes' (Orvig, 1968). Among jawless vertebrates and fishes, the differentiation of several dermoskeletal elements is controlled by neuromasts (sensory cells) associated with the cephalic and lateral-line canal systems (Pehrson, 1944; Devillers, 1947). This situation may be retained among archaic tetrapods but it is lost among all amniotes. In all tetrapods, the general dermal skeleton has lost all of its dental components except for those located around the mouth cavity and those forming the jaws and palate (dentigerous bones). The number of these components decreases through tetrapod evolution.

4. Dermal and membrane bones (French: os dermiques, os de membranes, os de recouvrement, German: Deckknochen, Nahtfaserknochen, pro parte). Dermal and membrane bones are the main bony components of the dermoskeleton, excluding teeth and scales. The term 'dermal bone' connotes the origin of the bone from the dermis, while 'membrane bone' emphasizes its direct ossification from an originally soft, unmineralized membrane. In most cases, dermal bones are somewhat flat with active radial growth primarily in one plane. Radial growth occurs peripherally within the unminalized fibrous sutures which delineate the border of the bone. Growth in thickness occurs on both the outer (superficial) and inner (lower, deep) bone surfaces through histogenetic process similar to those of periosteal ossification in the endoskeleton (Section III). On the other hand, sutural ossification commonly involves mineralization of pre-existing collagenous fibers deposited in the active sutures, and results in densely 'fibrous' bony tissues (the "Nahtfaserknochen" of Gross, 1934) with dense bundles of Sharpey's fibers (Section IV).

Changes in the radius of curvature of growing dermal bones are accomplished by differential deposition and
resorption on superficial and deep bone surfaces as well as within the interior vascular or sinusoid cavities of the bone. This internal remodeling produces characteristic textural patterns in the diploe, the more or less spongy, cancellous central region which separates the outer or superficial (sometimes ornamented) cortex from the inner, deeper one.

Patterson (1977) proposed to restrict the term 'membrane bone' to endoskeletal elements which differentiate directly during ontogeny without a transitory cartilaginous stage, and endochondral ossification (e.g. teleost vertebrae). He retained 'dermal bone' for the bony elements of the dermoskeleton. However, his suggestion has not been generally followed. If accepted, this would change the significance of terms used in many classical descriptions.

5. Long bones. This term emphasizes the general elongated shape and structure of bones considered as anatomical entities. Typically, long bones are more or less cylindrical and elongated along a major axis. They may vary extensively in shape and proportions, and all transitions may exist between them and 'short bones' or 'flat bones' (Figure 1B).

Long bones are, on the whole, best expressed in tetrapod limbs. In such cases, the long bone comprises a more or less cylindrical central **diaphysis** containing the marrow cavity, which is delineated by a superficial **cortex** generally consisting of compact bone. The cortex is a bony material deposited centrifugally via periosteal ossification (see Section III). The two extremities, or **epiphyses**, are capped by the articular cartilages, and consist of a spongyosa of cancellous bone derived from endochondral ossification.

Through the antagonistic processes of cartilaginous proliferation and endochondral ossification, the epiphyses are responsible for overall growth in length of the long bone along its main axis. It is noteworthy, however, that the relative contribution of the two epiphyses to overall growth can vary enormously from a well-balanced contribution to a nearly complete predominance of one epiphysis over the other.

Epiphyses are sometimes equated with the secondary centers of ossification which differentiate within the extremities of young, developing long bones, notably among mammals and lizards (see Haines, 1942). However, a current trend is to use 'epiphyses' in a more general anatomical significance as long bones extremities, and to refer to secondary (intra-epiphysial) centers of ossification when relevant (Haines, 1969; Ricéls, 1979; Francillon, 1981).

**Metaphyses** are commonly ill-defined transitional regions between the epiphyses and the diaphysis where peripheral reduction in epiphyseal diameter takes place. The original diameter of the epiphysis is larger than the diaphysis. However, a given transverse area of a growing epiphysis must eventually become relocated into the narrower diaphysis. This implies a local reduction in diameter through resorption (Enlow, 1963). In this transition zone, metaphyses commonly have a cortex with a characteristic structure of spongy bone of endochondral origin which is later converted into compact, coarse, cancellous tissues.

**Growth cartilages** (French: cartilages de croissance, cartilages de conjugaison). Growth cartilages are located between epiphyseal secondary centers of ossification (when present) and the region of endochondral ossification in the metaphysis. These cartilages disappear at adulthood among mammals, where linear growth ceases at a predetermined level. However, growth cartilage persists, in a poorly developed state, among adult lizards, where moderate growth in length remains possible throughout adult life. In vertebrate groups which do not develop secondary centers of ossification (crocodilians, chelonians, urodèles, etc.), there is a complete structural continuity between the articular cartilage forming the epiphyseal surface and the growth cartilage located deeper in the metaphysis.

6. **Short bones**. Short bones typically show stout, cuboidal or irregular shapes. They may occur within the dermoskeleton or endoskeleton, but they are more common in the latter. Endoskeletal short bones commonly differentiate and grow in ways similar to independent secondary centers of ossification in the epiphyses of long bones (Haines, 1942). Within an initially cartilaginous model, this endochondral ossification begins at the center then spreads radially outward in association with cartilage canals which contain blood vessels. Alternatively, short bones may develop in a manner similar to long bones as a whole (e.g. the vertebral centra), with two opposite endochondral cones united by a common cortex of periosteal bone. Like long bones, short bones commonly associate with **apophyses**. These are localized expansions of the bone which generally serve as sites of ligament or tendon insertion. They are initially independently ossified close to the bone, and they later fuse with its main body.

7. **Flat bones**. Flat bones show obvious preferential development within a single plane or a single curved surface. These bones differentiate both in the endoskeleton (especially in the limb girdles), and in the dermoskeleton (especially in the skull). On the whole, they are more common in the dermoskeleton. They lack typical epiphyses and free muscular cavities, but consist instead of a diploe, a central zone of cancellous bone sandwiched between two layers of compact cortex. In the endoskeleton, the development of flat bones is similar to short bones. But in the dermal skeleton, their growth involves fibrous sutures on the entire periphery or in specific regions of the bone margins. Apophyses are common. As it is evident in ribs, all intermediate morphological situations can exist between typical long, short and flat bones.

8. **Ornamented bone** (French: os sculpté, os orneménté) (Figure 2L). Bone ornamentation consisting of grooves and pits is commonly observed on the outer surface of the skull and osteocuts (ostecods) of poikiloothermic tetrapods. Ornamented bone usually consists of lamellar bone showing cyclical growth marks (Bürffénial, 1982). The nature of ornamentation is related to local rates of bone growth (Bystroff, 1935, 1947). In crocodilians, the ornamentation reflects differential resorption of the bony surface (Bystroff, 1935; Bürffénial, 1982). Although perhaps related to thermal regulation, the precise function of this ornamentation remains unknown.

9. **Accessory bones**. This heterogeneous morphological category includes various non-pathological ossified structures which are subordinate to the main morphological categories of bones, or 'cardinal bones', just reviewed. These bones are characterized only by a relative lack of constancy of location, size, shape and function.

In the dermal skeleton, one group of accessory bones is represented by the supernumerary flat bones in the skull, such as the wormian bone, inca bone, etc., which differentiate in sutural regions between the 'regular' bones. They may be of considerable interest for comparative anatomy because some represent rudiments of bones once
well developed in remote ancestors. In the endoskeleton, accessory bones are formed by elongated ossifications of ligaments and tendons, the latter forming a prominent feature of normal leg skeletogenesis in many birds. Similarly, the numerous intermuscular bones of bony fishes (French: arêtes) directly ossify within myosepta, the sheets of connective tissues lining each successive myotome.

More or less spherical or ovoid ossifications linked to the joints (periarticular regions), tendons and ligaments, such as the knee cap, or patella, are collectively called the sesamoid bones. These are especially well-known among mammals in the carpal, tarsal and phalangeal regions. Most of those small bones, as well as an ossified meniscus (accessory condensation of hard connective tissue associated with the knee joint), when present, are generally interpreted as progressive evolutionary developments appearing among specific lineages for biomechanical reasons (Lessertissier and Sahni, 1967).

Heterotopic bones (or endosteres) are isolated bones not linked anatomically to the other parts of the skeleton. Among mammals, well-known examples include the penian and elitoridian bones, as well as the heart bone(s) (ossa cordis), which is especially well developed among cervids and bovids. Other heterotopic bones formed under normal conditions are the carotidian fork bone (in horses), and bones in the diaphragm, oesophagus and even in the amygdales of various mammals.

B. Dermal Structures and Scales

1. Dermal sclerifications (French: sclérifications dermiques). Sclerification refers to the process of hardening biological tissues. Dermal sclerification (Bertin, 1958; Moss, 1969b, 1972), or the formation of intradermal reinforcing tissues, occurs in all vertebrates except birds. According to Bertin (1958) dermal sclerification includes modified scales in the osteichthyans, such as spines and scutes in teleosteans and osseous plates of chondrostians, holostean and teleosts. The term dermal sclerification is used (Moss, 1969b) for the great variety of reinforcing tissues which "are not all bone" in the dermis of reptiles.

2. Osteoderms. This term refers to mineralized plates in the dermis of amphibians (Anura), reptiles and mammals. Each osteoderm consists of a mineralized plate which may be vascular or avascular depending on the species (Gui bé, 1970). In some lizards, the osteoderms are isolated plates connected to one another by unmineralized collagen bundles (Romer, 1956). In the Anguidae, these plates are imbricated in the same manner as the elasmoid scales of teleost fishes, and there is an exact coincidence between these ossified dermal plates and keratinized epidermal scales (Otto, 1908). In other lizards, the osteoderms fuse to form a mosaic which does not correspond with the keratinized epidermal scales (Otto, 1908). Osteoderms are particularly well developed in the Crocodilia.

The term osteoderm is not clearly defined in turtles. In numerous turtle families, the dermoskeleton comprises a thecal shell (the primary bony armor; Zangerl, 1969) consisting of a mosaic of dermal bony plates forming two distinct parts: the carapace (the dorsal arched disc) and the plastron (the ventral flat plate). In families such as the Trionychidae and the Dermochelyidae which show a reduced shell, dermal 'ossicles' occur as isolated plates or they are associated in a mosaic. These epidermal plates or secondary armor (Zangerl, 1969) are probably homologous with the osteoderms of the other reptiles.

Reptile osteoderms consist of various types of mineralized tissues, some of which are not considered to be true bone by Moss (1969b, 1972). However, ultrastructural analyses show that osteoderms consist of different types of bone tissues, with the exception of globular mineralization which lacks a collagenous matrix (Zylberberg and Castanet, 1985; Levrat-Calvaci and Zylberberg, 1986; Levrat-Calvaci, 1987). Anuran osteoderms are considered to consist of a bony tissue; the organic matrix is composed of collagen fibrils organized in superimposed plies (Ruibal and Shoemaker, 1984). These osteoderms differ structurally from the ossified dermal scales of Gymnophiona amphibians. Moreover, compared with Gymnophiona dermal scales, the osteoderms differ ontogenetically in their formation by metaplastic ossification (Zylberberg and Castanet, 1985; Levrat-Calvaci and Zylberberg, 1986).

A coherent interpretation of the phylogenetic significance of osteoderms is difficult because the arrangement, shape, and structure of these mineralized plates vary not only among families but between species in the same family. Moreover, the osteoderms are not morphologically uniform within a given animal. Osteoderms appear to have independently arisen among the anurans and reptiles (Moss, 1969b; Ruibal and Shoemaker, 1984).

3. Scales (Figures 2 and 3; French: écailles; German: Schuppen). As presently used, the term 'scales' excludes the epidermal scales of birds, reptiles and mammals, which are keratinized structures belonging to the epidermal layer (exoskeleton proper). In the context of the present chapter, scales are mineralized elements which form in the upper part of the dermis, generally close to the epidermis. They differ from dermal bones which form in the deeper part of the dermis. Scales can regenerate after removal. Fish scales show great polymorphism: small tooth-shaped units occur in the Elasmobranchii; thick osseous plates occur in primitive Osteichthyans; and thin lamellar plates or thin bony scutes occur in Teleostei (Goodrich, 1907; Bertin, 1958). Elasmobranch scales are called placoid whereas those of the primitive Osteichthyans are called rhomboid. Among teleosts, thin lamellae scales are called elasmoid whereas the other varieties are known as scutes or spines (Meunier, 1983, 1984b; Sire, 1987).

The study of the ontogeny of rhomboid (polypterid) and elasmoid (teleost) scales permits new interpretations of their evolution (Géraudie et al., 1988). Rhomboid scales consist of two closely fused parts: a superficial part probably derived from odontodes and a deeper part probably derived from dermal bone (Orvig, 1968). Dentine and enamel, which correspond to the superficial parts of the rhomboid type, could be derived from fused odontodes. It is generally believed that dentine and enamel disappeared in elasmoid scales except in Latimeria, where obvious odontodes are inserted on the posterior area of the scale (Smith et al., 1972; Castanet et al., 1975).

Among amphibians, the Gymnophiona have dermal scales inserted within a scale pocket. Each scale is composed of various layers (Saras and Saras, 1887-1900; Gabe, 1971). The general organization of a gymnophionan scale might be compared with that of teleostean elasmoid scales. However, the squamation, which are the only mineralized part of the scale, and which form a discontinuous layer at the outer
Fig. 2. Elements of the dermoskeleton. A: Typical ctenoid scale of a teleost Hemichromis bimaculatus (Cichlidae). AF = anterior region; c = ctenii; F = focus; PF = posterior region; R = radius; T = tubercles. B-F: Elasmoid scales of various Osteichthyes, oriented as in Figure 2A. B: Gnathopemus petersoni (Mormyridae) with a network of grooves. C: Amia calva (Amiidae) with radial ridges. D: Cyprinus carpio (Cyprinidae), a typical elasmoid scale. E: Perca flavifilis (Percidae): a typical ctenoid scale. F: Salmo gairdneri (Salmonidae), a cycloid scale without radii. G: Dermal sclerification of Macrorhamphosus (Macrorhamphosidae). H: Dermal scale of Balistes (Balistidae). I: Spine of Chilomycterus (Diodontidae). J: Scales of Plecostomus commersonii (Loricariidae); as = anterior region; ep = epidermis; le = lateral-line canal; ps = posterior region with numerous odontodes. K: Dorsal scutes of Acipenser (Chondrostei). L: Ornamented bone; dorsal view of the skull of a stegocephalian amphibian showing ornamentation pattern of dermal bones; pits and grooves indicate regions of slow and active radial growth, respectively. Sources: A from Sire (1986); B-F from Meunier (1987); G-I, K from Hertwig in Bertin (1958); J from Goodrich (1909); L from Bystrow (1935).

surface, show globular mineralization (Zylberberg et al., 1980).

a. Placoid scales. These elasmobranch dermal denticles consist of an enamel or enamel-like (enameloid) layer covering a dentine crown enclosing a pulp cavity. These denticles also have numerous canaliculi radiating from the pulp cavity, and they may have a basal plate of acellular bone (in the Chondrichthyes). Like teeth, they form within dental papillae, through inductive interaction between the epithelium and the mesenchyme. They erupt early in embryos, sometimes before hatching, and they do not subsequently grow. They are merely replaced when shed
Fig. 3. Elements of the dermoskeleton (continued): rhomboid and elasmoid scales. **A:** Four rhomboid scales of *Porolepis* sp. (Crossopterygii) in natural position. **B:** Detail of an isolated scale of *Porolepis* sp. showing the narrow anterior overlapped region and the large, shiny ornamented posterior region. **C:** Anatomical relationships between rhomboid scales and skin in *Lepisosteus osseus* (Holosteii); scales are imbricated and covered by epidermis; *ap* = anterior articulating process; *sk* = scale; *skn* = skin covering scales. **D:** Longitudinal section of the tegument of *Polysterus* (Brachyopterygii) showing three scales linked to each other by collagenous fibers and overlapped by epidermis; *b* = osseous basal plate; *coll* = collagenous fibers; *d* = dentine; *e* = ganoin layer; *ep* = epidermis; *m* = muscles; *vc* = vascular canals. **E:** Elasmoid scale of *Holopterus* sp. (Crossopterygii). **F:** Anatomical relationships between elasmoid scales, skin and muscles in *Lepisosteus ctephalus* (Teleostei); the scales, inserted in the dermis, are extensively imbricated; *lsc* = lateral-line scales; *m* = muscles. **G:** Vertical section in the tegument of *Phoxinus phoxinus* (Teleostei) showing the imbricated scales in their scale pocket, obliquely inserted in the dermis; *ep* = epidermis; *hd* = hypodermis; *sp* = scale pocket; *stc* = stratum compactum of dermis.


**b. Rhomboid scales.** These primitive Osteichthyes scales are thick and juxtaposed, unlike the thinner, imbricated elasmoid scales. A rhomboid scale consists of a thick basal plate of parallel-fibered bone, and a superficial layer of vascular bone in addition to dentine and a hypermineralized substance (ganoin) (Williamson, 1849; Hertwig, 1879; Goodrich, 1907; Kerr, 1952; Ørvig, 1967; Schaeffer, 1977). Rhomboid scales are known from lower Devonian *Lophosteus*, the oldest known fossil representative of the Osteichthyes (Schultze, 1977). It is from rhomboid-type scales that cosmoid (Sarcopterygii) and ganoid (Actinopterygii) scales differentiated. The main differences in these types deal with the organization of the dentine and enamel.

Rhomboid scales have a dorsal peg which fits into a socket in the next scale. Rhomboid scales are connected to one another and to the dense dermis by Sharpey's fibers.

The scutes of the sturgeon (Chondrostei) have been interpreted as rhomboid scales in which dentine and enamel have disappeared.

**c. Cosmoid scales.** In cosmoid scales, a dense osseous basal plate is covered by a spongy layer with large vascular
spaces (Goodrich, 1907; Gross, 1935, 1956; Ørvig, 1969; Thomson, 1975, 1977). Above this spongy layer occur odontodentine derivatives associated with small chambers opening on the upper scale surface and contributing to a pore-canal system. This system, which is closely associated with dentine and enamel, organizes in isolated odontodes, has been called cosmine. The odontodes can be deposited in a succession of several layers surrounding a network of vascular canals (as in primitive cosmoid scales) or in a single layer on which the enamel is well developed. Cosmoid scales occur in certain Osteostraci and in numerous Classopispididae and Dipnoi. They do not occur in living fishes.

4. Ganoid scales. The term ganoid or ‘shiny’ was first applied by Agassiz (1833-1844) to a heterogeneous assemblage of living and fossil fishes, the taxonomic association of which has since been abandoned. Goodrich (1907) restricted the term ‘ganoid’ to the scales found in all Actinopterygii except the Teleostei. He divided these ganoid scales into palaeoniscoid and lepidospondylous types.

5. Dactylopteroid scales. These Palaeoniscus-type ganoid scales have three superposed layers: an inner layer composed of compact parallel-fibred bone, a middle vascularized layer of dentine, and a superficial layer of cosmine (= stratified enamel) (Goodrich, 1907; Stewertzépf, 1932; Aldinger, 1937; Daget, 1950; Gross, 1953, 1966; Ørvig, 1957; Schultze, 1966; Meunier, 1980b). In some scales, the network of vascular canals is well-developed, as for instance in polypterids. The palaeoniscoid scale is the first type of ganoid scale to appear in the fossil record, and it is apparently the basic type. The most ancient known example occurs in the Devonian palaeoniscoid Chondreptus. These scales grow by accretion of ring-shaped peripheral regions with new bone and ganoid layers. Palaeoniscoid scales occur in extinct Chondrostei and in extinct and living Brachiopterygi (= Cladistia), e.g. in Polypterus and Calamoichthys.

6. Lepisosteoid scales. Lepidopterygian-type ganoid scales lack dentine (Williamson, 1849; Nickerson, 1893; Goodrich, 1907; Aldinger, 1937; Ørvig, 1951; Schultze, 1966). The ganoid layer is directly deposited on the surface of the osseous plate. The parallel-fibred bone may contain scarce vascular canals and numerous fine tubules; canals of Williamson are present. These scales occur in the living Lepisosteus and Arapaima, and in numerous extinct Holostei.

7. Elasmoid scales. Elasmoid scales are transparent, thin, lamellar, imbricated osseous plates (Bertin, 1944). They consist of two main layers: a thick, lamellar, partially mineralized basal plate and a plywood-like structure (= isopedin), covered by a thin, ornamented superficial layer (the external layer, or ‘osseous layer’). In the posterior region, the scale is covered by the epidermis and an outer limiting layer is deposited on the surface of the external layer. These scales are localized in a pocket (see scale-pocket and scale-sea in Section 1). Elasmoid scales generally do not show fibrous links (collagenous fibers) with the surrounding connective tissue. Elasmoid scales occur in most Teleostei and in the Amiidae in the Acanthopterygii, but also in Latimeria and in the Dipnoi in the Sarcopterygii (Meunier, 1983). In some teleostean families (Callichthyidae, Gasterosteidae, etc.) elasmoid scales are replaced by other dermal formations (see scutes, below).

The elasmoid scale type was subdivided into cycloid and scuteloid scales by Goodrich (1907). However, this classification is based only on the presence or absence of comb-like structures or crenii on the posterior region of the scales, and it is artificial and non-representative of the diversity of structure and organization of elasmoid scales in the Osteichthyes (Kobayashi, 1952-55; Meunier, 1983; Sire, 1987). The cycloid scale type is rather homogeneous and includes the scales of numerous ‘evolved’ Teleostei (e.g., the Perciformes), but the cycloid type requires revision and subdivision. Some cycloid scales are very close to ctenoid ones and differ only in their absence of ctenial spines, whereas other cycloid scales are very different. For example, the scales of the Osteoglossiformes have polygonal squamation separated by a reticulated network of grooves (Meunier, 1984a), scales in the salmon lacks radii, and those of some Teleostei except the Teleostei. He divided these ganoid scales into palaeoniscoid and lepidospondylous types.

4. Squamulae. Squamulae are mineralized plates forming a mosaic at the outer surface of dermal scales in the Osteichthyes and Amphibia (Gymnophiona). In fish scales, the outer surfaces of squamulae are ornamented with denticles or tubercles in the Dipnoi (Kerr, 1953a; Meunier and François, 1980; Zylberberg, 1983), and in the Osteoglossidae (Cockerell, 1911; Meunier, 1984a,b). Squamulæ are flat in the eel (Zylberberg et al., 1984). In the Gymnophiona amphibians, the superficial ornamentation of the squamulæ varies among the species, as demonstrated in Taylor’s atlas (1952). This author pointed out the utility of scales in classifying the Gymnophiona.

5. Scutes (= bony plates, transformed scales). Scutes are dermal sclerifications composed of osseous tissue which shows a great diversity of shape and organization, ranging from large bony plates of acellular bone surrounding vascular cavities (in sturgeon, armored catfish, Agonidae, Ostracodontidae, and Molidae); to round or elongated plates with a well-developed network of cavities (in Thunnus, Makaira and Ruvettus); and spine-like formations (in Tetradontidae, Diadontida, etc.). Precise homologies between scutes and typical elasmoid scales are generally unknown (Bertin, 1958; Whelan, 1986). Scutes, 'scutes' or 'osteoscuta' occur as bony plates in the skin of reptiles or mammals. Osteoscuta are thick bony plates in the dermis, and this term is often used as an synonym of 'osseouscuta' (keratinous epithelial dermal scales). See also osteoderms (above).

6. Spines. Like scutes, spines are modified elasmoid scales. Spines show a continuous spectrum of stages from the typical elasmoid scale to thin spines, as observed in two families, the Diodontidae and the Tetradontidae (Bertin, 1958).

7. Tesserae (= tessellae). Tesserae are either small plates of dermal armor in fossil osteoderms and placoderms (Haleatop-Tario, 1967; Haleatop-Tario, 1973) or prisms or small blocks of cutaneous tissue belonging to the cartilaginous endoskeleton of elasmobranchs and holoccephalans (Ridewood, 1921; Appleby, 1967). Moss (1977) recommended using ‘tesserae’ for fossil fish dermal bone tissue only to avoid unnecessary confusion between dermal skeleton and calcified cartilage. Kemm and Wernert (1979) favor the term 'endoskeletal tesserae' for the calcification in sharks, because of its descriptive significance and its long
use by paleontologists and histologists (Ridewood, 1921; Ørvig, 1951; Applegate, 1967; inter alia).

C. Teeth-like Structures

1. Odontodes (dermal teeth, dermal denticles; Figure 4). Defined first by Ørvig (1967) as hard tissue units of the skin, odontodes correspond closely with simple teeth, and they are difficult to distinguish from true teeth by any rational criterion. However, odontodes belong with non-dental parts of the dermal skeleton, and they do not fulfill similar functions as teeth (Ørvig, 1957, 1977, 1978). Odontodes function mainly for protection.

An odontode consists of a core of dentine or denitinous tissue (mesodontine in acanthodians; semidentine in arthrodiras) surrounding a pulpar cavity. They commonly also possess a superficial cap of enamel or enameloïd. Vascular supply occurs through basal canals. The base of the odontode consists of cellular or acellular bone which functions as an attachment tissue. The formation of an odontode takes place in a single, undivided dental papilla of mesenchymal tissue always located in the superficial part of the cornum (or dermis vascularized connective tissue). It is bounded at its outer surface by an epithelial dental organ which lacks the complicated structure of tooth germs. It consists of a single layer of turgid, columnar cells in the basal epidermis. Odontodes are well known in the placoid scales of selachians, in the Osteostraci, Anaspida, Placodermi, and many primitive osteichthians. They are likely the earliest constituents of the dermal skeleton. They are still seen among living species of Latimeria and Polypterus. Odontodes develop independently as tooth units on the surface of the dermal skeleton in Lepisosteus, Polypterus, and in some Siluriformes (Calfichthysidae, Loriciaidae) (Bhaut, 1938).

2. Odontocomplex. This was defined by Ørvig (1977) as an agglomeration or cluster of odontodes forming directly upon one another or beside each other during consecutive stages of growth. This occurs in acanthodians, polypterygids, lepisosteids and many other fossil groups.

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**Fig. 4.** Elements of the dermoskeleton (continued): odontodes and odontocomplex. A: Odontodes (O) on a fragment scale (X) in *Plecostomus commersonii* (Loricariidae). B: Scales of *Latimeria chalumnae* with several generations of odontodes on their posterior region. C: Vertical section of an isolated odontode of a *Latimeria* scale; above an ossous basal plate is a simple pulpar cavity lined by dentine, which is overlapped by a thin cap of enamel or enameloïd. D: Detail of the superficial part of a vertical section in a *Latimeria* scale showing two successive odontodes lying on the ornamented layer. E: Diagram of an odontocomplex consisting of three odontodes; it could include a fourth, fifth, and so on, by farther growth: ep = epidermis; g1, g2, g3, = granular layers of the first to third odontode of the complex; t = resorption line; t1, t2, t3 = fully developed odontodes of the complex. F: Three successive stages of development (from right to left) of a placoid scale in the dogfish *Scyliorhinus canicula* (Chondrichthyas); d = dentine; e = enamel or enameloïd; ep = epidermis. Sources: A-F from Goodrich (1909); B-D, E from Ørvig (1977); C from Roux in Ørvig (1977).
D. Fish Rays

1. Fin-rays (dermotrichia; Goodrich, 1904). Fin-rays are dermoskeletal elements supporting fish fins. These include bony spiny rays and bony lepidotrichia found in all the osteichthyans except the Dipnoi, the camptotrichia present only in the Dipnoi, the non-mineralized ceratotrichia of the Chondrichthyans, and the non-mineralized actinotrichia of the Osteichthyans.

2. Spiny rays. These are the unjointed and distally pointed bony rays found in the anterior portion of some fins (especially pectoral, first dorsal and anal) of some teleosts (e.g., Siluriformes and Perciformes). Typical spiny rays (Perciformes) are unpaired bony thorny needles, the distal extremity of which are devoid of actinotrichia (see below). They are not capable of regeneration after injury (Blanc, 1950). In the Siluriformes and in the Cyprinidae, the spiny rays are transformed lepidotrichia which show an important centrifugal osseous accretion (Vaillant, 1895a,b).

3. Lepidotrichia (Figure 5). This term was proposed by Goodrich (1904) to designate the osseous fin rays of the Acipenseriformes. A lepidotrichium consists of successive adjacent bony elements connected to one another by ligamentum. Each consists of two parallel rows of elements in the form of a parenthesis ( ), called a hemisegment by Laning (1926), a demibranch by Kemp and Park (1970), and a hemilepidotrichium by Géraudie and Landis (1982). Consequently, it is possible to split a lepidotrichium longitudinally into two equivalent parts. It is made of either cellular or acellular fibrous bone (Moss, 196a, 1972), in agreement with the general bone type of the whole skeleton.

In the fins of the Brachipterygiiformes (Pleuropteriidae), a patch of ganoine incompletely covers the upper surface of the most proximal segments of the lepidotrichia (Géraudie, 1988) as in the Lepisosteidae (Hertwig, 1879; Nickerson, 1893). The most proximal segments consist of cellular fibrous bone. In all Osteichthyans, lepidotrichia are capable of regeneration following injury.

4. Camptotrichia. This term was coined by Goodrich (1904) for the fin rays of the Dipnoi which do not resemble lepidotrichia, although they perform the same function (Géraudie and Meunier, 1982). Camptotrichia are simple, long irregular cylindrical rods with a tapered distal extremity. They are adjacent parallel units that branch distally, are disposed in two rows in the fins, and are not symmetrical. Camptotrichia eventually present, formed by a swelling of the rod where there is an interruption of mineralization.

A camptotrichium consists either of cellular fibrous tissue (in Neoceratodus) or acellular tissue (in Protopterus) which mineralizes only on the subepidermal region of the rod. The deep (dermal) region of a camptotrichium is not mineralized (Géraudie and Meunier, 1984). Consequently, only the subepidermal region of a camptotrichium is considered to be typical fibrous bone; its deeper region has been interpreted as a bone derivative which has lost the ability to mineralize, and consequently has become a permanent pre-osseous tissue (Meunier, 1987a).

It has been hypothesized on the basis of paleontological studies of fossil fish fins (Jarvik, 1959) that camptotrichia evolved from completely ossified fin rays similar to lepidotrichia. Camptotrichia may therefore represent transformed lepidotrichia which lost their symmetrical arrangement. Speculating further, a camptotrichium may be homologous to an homolepidotrichium.

Comparative studies of lepidotrichia and camptotrichia suggest that lepidotrichia represent a plesiomorphic (primitive) character in the fins of all Osteichthyans and consequently that the camptotrichia of only the Dipnoi could be apomorphic (derived).

5. Ceratotrichia. These "horny" translucent, tapered rods were first described by Kruekenberg (1880) for selachian fins. They are longer (several cm) and larger than actinotrichia, which are considered to be homologous with ceratotrichia. Apart from the scales, they constitute the only dermal skeleton in selachian fins. Ceratotrichia consist of elastoidin, and they are the basic source of the material from which biochemical studies on elastoidin are carried out (see Section VB1d, below).

6. Actinotrichia. The term "actinotrichia" was used by Ryder (1884) to designate the "hairy" elements located at the distal free edge of the lepidotrichia of a fin. The adipose fins of some Salmonidae and Siluridae contain exclusively actinotrichia. Actinotrichia are slender and short (a few mm long), unmineralized tapered rods, eventually distally branched. They are observed between the most distal hemisegments of the lepidotrichia in all Osteichthyans including the Coelacanth (Géraudie and Meunier, 1980). They are capable of regeneration after fin injury.

Actinotrichia are made of elastoidin and are thought to be homologous with the ceratotrichia found in the Chondrichthyans (Goodrich, 1904).

Actinotrichia have been observed inserted within the bony substance of camptotrichia (Géraudie, 1984) and lepidotrichia, where they fade (Goodrich, 1904; Géraudie and Landis, 1982; Géraudie, 1988). This morphological disposition suggests that actinotrichia may mineralize after structural and biochemical modifications of the elastoidin which are not yet understood.

III. The Microanatomical Level: Bone Structures And Processes

This section deals with a level of morphological integration intermediate between organ and tissue, i.e., between the gross anatomical level, which is readily studied with the naked eye, and the study of the component tissues themselves. Best observed with the binocular microscope, at lower magnifications with the compound microscope, and with SEM, this microanatomical level deals with the fine structural organization of bones as organs. This level deals with particulars such as porosity, vascularization, and the fine spatial arrangement of the major sub-components of bone.

From a functional point of view, this level of observation offers clues regarding how the shape of bones are attained, maintained or changed during growth. This level also deals with the biomechanical demands imposed upon bones and it offers a general perspective on how fine tissue level mechanisms operate to shape an organ. Some of these topics have been introduced in the preceding section. They are discussed in greater detail through the following definitions of structures and processes.
Fig. 5. Fin dermoskeleton. **A**: Sequence of development of the fin dermoskeleton. First actinotrichia (a) develop in the fin bud, then bony lepidotrichia (l) differentiate directly in the dermo-epidermal interface boundary of the skin. **B**: Dermoskeleton of the fin; actinotrichia (a) and lepidotrichia (l) are connected with endoskeletal elements (r) and muscles (m); note that elasmoid scales do not cover the fin. **C**: Three-dimensional reconstruction of a teleost fin. **Sources**: A from Géraudie and Landis (1982); B from Goodrich (1904); C from Becerra et al. (1983).
A. Structures

1. Long bone organization

a. Epiphysis. This term was used by Haines (1942) for the entire mass of cartilage at the end of long bones as far as the region of endochondral ossification, which is functionally dependent on it. Secondary centers of ossification developed in the cartilage will be referred to as such. In the early literature, the word 'epiphysis' was used almost exclusively for these bony centers. Depending on the vertebrate group, the epiphyses are differentiated as secondary centers of calcification (in some Chondrostei and Dipnoi). Epiphyses show more or less regular arrangements of the cells of the growth cartilage (in the Crocodilia, Chelonia, and Urodela), bony secondary centers (Lacertilia), and cartilage canal (in some Repulida and Mammalia).

b. Metaphysis. In a long bone the metaphysis is the transitional region between the epiphysis (or the transverse trabeculae which mark its limit in the cancellous osseous tissue of the adult) and the diaphysis, i.e., the shaft proper (Lacave, 1949). An autonomic 'metaphysis' is best applied to growing bones because it is directly linked to the growth cartilage from which it originates (Rubin, 1964). This is why the structure and composition of the metaphysis distinguish it from the diaphysis, even though it forms a prolongation of the diaphyseal shaft.

Microangiography of the growing skeleton in several species shows that the same metaphyseal organization is present in long and short tubular bones as well as in many other bones such as the pelvis, scapula, cuboid bones like the calcaneum and talus, and cartilaginous bones at the base of the skull (Balmain et al., 1983).

c. Encoche d'ossification. This term, coined by Ranvier (1873), is generally used in the French form even in the English and German literature for the extremity of periosteal bone which develops around metaphyseal regions of actively growing young bones. The encoche d'ossification corresponds to the region where the once present perichondrium changes histologically and functionally into a perivascular which begins laying down new bone. Because longitudinal sections show the bone apparently sticking into the perichondrium as a spine, it has been given this special name ('encoche' means notch). The encoche d'ossification precisely follows the cartilage growth in length, hence it is always located at the periphery of the ferried chondrocytes in the metaphyseal cartilage growth plate (Haines, 1942, 1969; Frandillon, 1981).

d. Diaphysis. This is the cylindrical part of a long bone which has a more or less constant diameter. Generally, the diaphysis is produced by an initial perichondral layer of bone around the cartilage. This layer thickens externally through periskeletal centrifugal ossification. Cartilage erosion begins in the middle part of the diaphysis and proceeds in the direction of the epiphysis. If the erosion is incomplete, a fine layer of cartilage may persist at the surface of the medullary cavity (Kutschereiko, 1881). The inner part of the diaphysis is a layer of endosteal bone.

e. Apophysis. An apophysis is a local protrusion of a bone which extends outwards some distance (Haines, 1942, 1969). Ontogenetically, apophyses commonly appear as independent ossification centers which develop close to the bone, with which they ultimately fuse. This accessory ossification center may involve either an epiphysis-like structure and endochondral ossification, or ossification of a pre-existing dense collagenic structure (tendon or ligament). Histologically, apophyses tend to show specialized tissue patterns related to their function as anchor for powerful tendons.

f. Primary and secondary centers of ossification. An ossification center is an inner region of a cartilage model which becomes invaded by blood vessels, and where early morphological changes of the chondrocytes occur as a preliminary to endochondral bone deposition.

In the long bones, the primary center of ossification generally refers to the center of the diaphysis. It is distinguished from secondary centers of ossification, which may develop in the epiphyses much later (Haines, 1942) (Figure 9).

g. Compacta and spongiosa (= compact bone and spongy or cancellous bone, respectively). These are two macroscopic categories of bone architecture which correspond to the first order structures proposed by Petersen (1930) and refined by Amprino and Godina (1947) and Riehl (1975a). The distinction between compacta and spongiosa is related to the overall bone porosity. Porosity can vary gradually from perfectly compact bone without holes (e.g., the avascular and acellular bone of some fishes) to bone with a free medullary cavity with only one or two trabeculae. However, in compacta the volume of bone tissue is higher than the volume of pore space and the converse is true for spongiosa (Parfit, 1983).

It is important to stress that the concepts of compacta and spongiosa involve neither precise histological structure, definite anatomical situations, nor ontogenetic origin. For instance, although a bone 'cortex' (the peripheral part) may generally consist of compact bone, cortex not composed of compacta also exists (e.g., the diaphyseal cortex of long bones in marine turtles and compacta also occurs outside the cortical bone, invading irregular regions (Riehl, 1975a)).

Because of metaphyseal reduction (e.g. during long bone growth), cancellous bone can be converted into compacted coarse and compacted fine bone as described by Enlow (1963). Conversely, compact bone, irrespective of pathological conditions, can be transformed into spongy bone by increasing the size of its vascular cavities (e.g., in the cortical diaphysis of long bones in marine turtles), and in area of the endosteal margin around the medullary cavity in many fossil amphibians and reptiles.

According to its degree of porosity, spongiosa has been subdivided into fine cancellous bone, coarse cancellous bone and trabecular bone, from lower to higher porosity (see below). 'Alveolar bone', i.e., the bone surrounding the root socket of a tooth, consists of both compact and spongy bone (Baron, 1972). Spongiosa can alternatively be classified according to its histogenetic origin. Riehl (1975a) distinguished primary and secondary spongiosa, each with subordinate categories. Secondary spongiosa, the more common category, results from remodeling primary spongiosa. Both types may be present in cortical as well as endosteal-endochondral bone.

h. Cortex and medulla. These terms refer to topographical aspects of bone architecture. The cortex, or cortical region, is simply the outer, peripheral part of a long bone, whereas the medulla is the inner, or deep central part (Enlow, 1963). These descriptive terms do not connote any information about the porosity or precise histological structure of the cortex and medulla. However, these terms are often confused with compacta and spongiosa because the
cortex is commonly compact in texture whereas the medulla is generally spongy. However, as noted previously, this is not always the case. Cortex can consist of fine cancellous bone and medulla can sometimes consist largely of secondary compacta (B4 in Figure 15).

i. Cortical stratification (Figure 6A). The cortex often consists of distinctive types of tissue, each forming stratified territories. These stratified patterns preserve a record of the local history of bone growth and morphogenesis. As noted by Enlow (1963): "compact bone is a mosaic of structure produced by the accumulation of growth stages and remodeling changes". Cementing lines are generally the most important features contributing to cortical stratification.

According to Enlow (1963), cortical stratification refers only to layering which reflects the remodeling and the juxtaposition of different types of bone tissue. Therefore, stratified structures within a given type of bony tissue (e.g. within lamellar or lamellar bone) are not considered to be "cortical stratification". The same applies to "growth zones" separated by test lines within the cortex of a long bone.

j. Trabecular bone (Figure 6D). Trabecular bone is a type of cancellous bone in which trabeculae show a precise three-dimensional spatial arrangement which reflects mechanical forces acting on the bone. Some trabeculae are oriented according to tensional and others according to compressional forces. This is the basic for the famous "trajectorial theory" of Wolff (1892), also known as "Wolff's law" (Murphy, 1936; Evans, 1957; Enlow, 1963, 1968). In a classical synthesis, Murray (1956) modified the trajectorial theory from its originally more rigid form. Murray noted that transformations of trabeculae between the embryonic and adult stages explain the fact that in the epiphyses of long bones, the trabecular architecture is not in harmony with that of the diaphysis. Finally, as now largely accepted, Murray (1956) emphasized that even though "in the determination of bony structures a very large part is played by mechanical forces and that "bony structures are in a general way mechanically adaptive", nevertheless mechanical constraints (compression, tension and shear) are not, independently of disease, "the only factors which influence the development of bony architecture". For extensive reviews and additional experimental works in this field, see Evans (1957), Hall (1965), Currey (1968, 1984) and Frost (1988).

2. Scale Structures

a. Cosmine (Figure 7A). The term "cosmine" was coined by Williamson (1849) and revised by Ørvig (1969) to refer to a combination of enamel (or enameloïd), dentine and trabecular bone around a pore canal system (Gross, 1930, 1933, 1956; Ørvig, 1969; Miles, 1975; Thomson, 1975, 1977; Meinke and Thomson, 1983). Cosmine characterizes cosmoid scales. Individual cosmine sheets consist of a great number of small, hard tissue units or odontodes (mesodentine in the Ostoasteridae, dentine in most other bony fishes). Cosmine also occurs in osteoid Rapididostom, early Dipnoans and Crossopterygians. The function of cosmine is unknown. It may have housed an electroreceptor organ (Thomson, 1975).

b. Scale surface and ornamentation (Figures 2-4, 7). The anterior and posterior regions of elasmoid scales are defined by the anterior margin of the epidermal cover which remains on the scale surface when the scale is pulled off (Baudela, 1873). These two regions are differently ornamented as follows:

Anterior region (anterior field; rostral field; overlapped field): This is the anterior part of the scale which is deeply inserted within the dermis and is covered by adjacent and more anterior scales. In most elasmoid scales this region is characterized by regularly disposed cirrii (defined below).

Posterior region (= posterior field; overlapping field; free region; caudal field): This is the exposed part of the scale which is covered only by a thin layer of loose dermis and by epidermis. In ctenoid scales, this region is characterized by ctenii (defined below) located at the margin of the scale (Kobayashi, 1952-55). The ornamentation of this posterior region shows great structural variety as observed by SEM (Dalmatier and Courtenay, 1974; Hughes, 1981; Sire, 1986).

Cirrulus (plural: cirruli; ridge, selerie, stria). A cirrulus is an elevation of the external layer of an elasmoid scale which forms a regular ridge at the surface. Cirri are generally concentrically deposited around the center (focus) of the scale but radial arrangements also occur (Baudela, 1873). In the latter case it is preferable to call these radial ridges. Cirri are more numerous in the anterior region of the scale, where they are commonly interrupted by radii. In the anterior region, the crests of the cirruli (dentate edges) can be ornamented with thin denticles which are not homologous with teeth. The cirri and their denticles anchor the scale in the scale-pocket. Cirri and their denticles do not change once their formation is complete and their characteristics can be used for systematics (Lanzing and Higginbotham, 1974; Sire, 1986). In the posterior region of the scale, the cirri are commonly progressively covered by tubercles, which represent thickenings of the outer limiting layer. The number and spacing of the cirri have been used to study growth and aging in fishes. This "scallometry" is based on the examination of annuli or growth marks.

Radius (plural: radii; radial groove). A radius is a radially oriented groove, generally issuing from the focus of a scale, characterized by the absence of the superficial layer (external). This radius is less than 10 per scale and are commonly localized in the anterior region. In the Cypriniformes, the radius are scarce (less than 10 per scale) and are found in different regions of the scale surface. In the Osteoglossiformes and Dipnoi, the scale surface shows both radially and concentrically oriented grooves which delimit the squamation (Meunier, 1848b).

Tubercles. "Tubercle" is a general term used to describe two kinds of elevations of different sizes at the scale surface:
1) Elevations on the posterior region of the scale which involve the outer limiting layer around all the anchoring buds. These tubercles develop throughout the life of the fish and they constitute a thick network (Sire, 1985b; 1986).
2) Small, round elevations of the surface of the ganoine of the dermal skeleton in various primitive Actinopterygii (polypteriids and lepisosteids). These tubercles probably serve to "anchor" the epidermis to the ganoine surface (Chabot et al., 1985; Meunier et al., 1988) and they are useful in systematics (Gayet and Meunier, 1986; Gayet et al., 1988).

Ctenius (plural: ctenii). A ctenius is a comb-like row of spines disposed on the posterior edge of a ctenoid scale.
Fig. 6. General organization of bone and growth patterns. A: General cortical stratification in a bone; cross-sections commonly show the superposition of different bone tissues forming the cortex which records the past history of local bone deposition, resorption and drift; in this drawing drift proceeds toward the right; 1: older bone cortex resorbed superficially on the left; 2: new apposition of periosteal bone; 3: remodelled cancellous bone; 4: apposition of endosteal bone. B: Growth remodeling of a long bone; four stages of growth show the regions of bone apposition (+) and resorption (-); sequential resorption (in black) and redeposition are normal
(Baudelot, 1873). The word ctenius has been incorrectly used for an individual spine. It is better to use 'ctenial spine' to refer to a single element of a ctenius. The last (most posterior) ctenius consists of newly formed elongated ctenial spines which protrude from the edge of the posterior margin of the scale. Ctenii may have a hydrodynamic function (Burdak, 1979), and they may be useful for conserving the laminar nature of water flow in the boundary layer on the body surface.

Ctenial spine (= denticulation). Ctenial spines are spines on ctenial scales which are approximately radially aligned relative to the scale focus. A ctenial spine consists mainly of a well-mineralized collagenous matrix; the central region shows fibril directions which are perpendicular to the long axis of the spine, whereas the peripheral region shows fibril directions parallel to the long axis. Ctenial spines insert on the scale surface (external layer) by means of a ligament-like unmineralized basis. This flexible basis allows for movement of the ctenial spine. When a new spine forms in a row, the preceding spine in the same row is progressively resorbed. The shapes of ctenial spines have been used in systematics (Hughes, 1981).

c. Basal plate (French: plaque basale; = lamellar layer or fibillary plate in elasmoid scales). The basal plate is the deep, largest part of a scale. In rhomboid (cosmoid and ganoid) scales, the basal plate consists of parallel-fibered bone, whereas in elasmoid scales it consists of a plywood-like structure called isodin. Basal plates are generally considered homologous among the various groups of fish (Schulze, 1977; Meinier, 1983).

d. Superficial layer (French: couche superficielle, couche côte; = ornamented layer, osseous layer, ridge layer). This is the upper part of an elasmoid scale, as opposed to its deeper basal plate. In elasmoid scales the superficial layer is divided into the external and outer limiting layers, defined below.

External layer. This name was used by Schönborn et al. (1979) for the thin osseous layer deposited first during ontogeny and covering all the surface of an elasmoid scale (Waterman, 1970; Lanzing and Wright, 1976). Circuli ornamenting the scale surface are elevations of this layer. Once deposited by superficial scleroblasts, the external layer does not thicken during subsequent growth of the fish (Sire and Meinier, 1981; Sire, 1985b).

Outer limiting layer. Defined by Schönborn et al. (1979), this is the most superficial layer of an elasmoid scale which is deposited after and above the external layer. It is often localized to the free region of the scale covered by epidermis. This well-mineralized layer differs from the external layer in being nearly devoid of collagen but rich in mucous substances (Zylberberg and Nicolas, 1982). This layer is deposited cyclically throughout the life of the fish; it develops preferentially around anchoring fibers binding the epidermis to the scale surface. This accumulation forms the tubercles clearly visible on the scale surface. The epidermis might actively participate in the formation of the limiting layer (Sire, 1985b). See also Zylberberg and Meinier (1981) and Sire (1985b).

3. Special or Pathological Conditions

a. Osteopenia (also spelled 'osteopenia'; French: ostéopénie, rarefaction osseuse). This term generally connotes a not necessarily pathological decrease in the amount of bone without any change in the normal amount of mineral per unit volume of tissue. Osteopenia implies thinning of cortical compact bone and/or of the trabeculae forming the spongiosa, as well as a reduction in number of trabeculae (Laval-Jeantet and Calain, 1981). The situation is well known in elderly humans, where the rate of bone loss after 50 years is about 0.3 % per year in men and 0.8 % per year in women. Skeletal adaptations of most flying vertebrates can in part be described as physiological osteopenia.

Osteopenia does not necessarily involve osteomalacia or osteoporosis (see below). Osteoporosis is pathologically severe osteopenia in which the reduction in bone volume

components of bone growth process and explain many peculiarities of cortical stratification. me = medullary cavity. C: Metaphyseal resorption; stippling = bone laid down between two stages of growth by endochondral ossification below the growth plate; cross-hatching = the amount of bone which has been eroded away as a result of growth remodeling. D: Trabecular bone, as organized in the proximal part of the human adult femur apparently agrees with the main directions of mechanical strain (tensive and compressive forces); a mechanical model (Kulmann's 'crane') shows grossly similar patterns. E: Growth process in flat bones; lateral growth mainly takes place at sutures between neighboring bones; 1: new bone forming in the suture pushing apart the already ossified material; 2: appositional growth takes place at the outer surface while resorption is performed at the inner surface and in the cancellous spaces; in that way, the thickness of bone is maintained commensurate with overall bone growth. F: Growth remodeling in flat bones; as a given flat bone grows, it may have to change its radius of curvature, i.e., according to overall skull growth as shown on three growth stages; this is realized by bone remodeling (erosion and new bone deposition), which accounts for local flat bone cortical stratification. G: Growth of short bones; three stages (1, 2, 3) are shown; 1: the bone model is still cartilaginous and only a core of endochondral bone develops in its center; the cartilaginous model grows both by internal expansion and subperichondral apposition at its periphery; 2: since endochondral ossification of the core proceeds faster than cartilaginous growth, a greater proportion of the model is replaced by cancellous bone; 3: finally, the model has been completely replaced by cancellous bone of endochondral origin, except in regions where superficial articular cartilages are left; growth has now ceased. Sources: A, B modified from Ricqlès (1979); C from Lesserissieux and Saban (1967); D,E,G from Sinclair (1973).
Fig. 7. Histological structure of rhomboid (A–C) and elasmoid (D–G) scales. A: Three-
dimensional reconstruction of cosmoid scale from Megalichthys. B: Three-
dimensional reconstruction of a palagonoid scale from Polystethus. C: Three-dimensional reconstruction of a liposaooid scale from Lepisosteus. D: Vertical section of an elasmoid scale from Hemicromis bimaculatus. E: Detail of the margin of an elasmoid scale of Carassius auratus with associated cells and mineralized regions (black and dark grey dots). F: Detail of three layers of an elasmoid scale from Carassius auratus showing the TC fibers that cross the collagenous plices of the fibrillary plate. G: Detail of an anchoring fiber in the scale of Hemicromis bimaculatus; the anchoring fiber links the external layer of the scale to the basal membrane of the epidermis. Legend: AB = anchoring bundles; AN = anterior margin; BP, BP = basal plate; C = canal; CE = external layer; CF = mineralizing front; ch = cavity of the pore canal system; Gl = cirrus; CM = Mandl’s corpuscles; Co = Osicular layer; d = odontode; Dd = dense dermis; Di = loose dermis; dt = dentine; E = epidermis; El = external layer; es = episcuama; EX = external layer; F = focus; Fa = anchoring bundles; Fm = mineralization front; FP = fibrillary plate; g = enamel; ga = ganoin; h = horizontal vascular canal; hs = hyposquama; J = epidermo-
dermal junction; LD = loose dermis; Le = outer limiting layer; me = Mandl’s corpuscles; OL, OL = outer limiting layer; oz = osteoid zone; Pd = basal plate; pe = pulpar cavity; Post = posterior margin; S = scleroblast; SFC = scale forming cells; ss = scleroblast; t = canal of Williamson; TC, y = TC fibers; VA = vascular cavity; Ve = vertical canal. Sources: A–C from Goodrich (1909); D from Sirc (1965b); E from Schönborn et al. (1979) in Whiten (1980); F from Zylberberg and Nicolas (1982); G from Sirc (1988).
exceeds the physiological limits of osteopenia for a given age class. This reduces the biomechanical safety factor of the skeleton under strain and hence osteoporosis is commonly - but not necessarily associated with fractures of the long bones or vertebral column (Rasmussen, 1980). Physiological osteoporosis, produced by intensive osteoclastic activity in cortical bone, is known in deer long bones and is linked with metabolic calcium demand during antler histogenesis (Goss, 1983). The ‘medullar bone’ of doves and several other birds similarly stores calcium for eggshells, and this bone may be extensively thinned and destroyed as a consequence of egg laying.

b. Osteomalacia (= rickets, pro parte). In mammals, osteomalacia is a pathological condition in which the amount of mineral per unit volume of tissue is distinctly below normal physiological values. The volume of bone matrix (mainly collagen) is normal in compact or spongy tissues, but this matrix is poorly mineralized. This condition is generally systemic, being found in dermal and endoskeletal tissues, and has especially drastic consequences during the growth of young individuals, including more or less extensive pathological deformation or skeletal fractures (rickets). Extensive clinical research in this field has demonstrated that vitamin D complex, numerous endocrine factors, environmental factors and diet are all related to the control of osteomalacia. It is noteworthy that, especially in bony fishes, the osteoid (young bone collagenous matrix) may have its mineralization considerably delayed, or even permanently postponed, sometimes leading to non-pathological ‘permanent osteoid or pre-osseous tissue’ (Meunier, 1987a). Even in mineralizing scales, mineralization may show an extensive time lag after the deposition of the fibrillar material by osteoclasts. This circumstance offers a useful model for studying the early phases of bimineralization of collagenous matrices.

c. Pachyostosis (also spelled ‘pachyosteosis’). This term simply indicates especially thick or massive bones. However, the significance of this term varies widely in the literature because it has been applied to various levels of integration and it has been used in various ways to explain bone peculiarities (Kaiser, 1960, 1970). Two applications of this word are explained as follows:

1. As a descriptive tool at the anatomical level (excluding obvious pathological conditions), pachyostosis is a relative term which can be defined only by intraspecific or interspecific comparison. Relative to a ‘normal’ condition, a pachyostotic dermal bone is thicker and a pachyostotic long bone is wider. This condition is especially obvious in the ‘banana’ ribs of many water dwelling tetrapods such as Mesosaurus (Paleozoic reptiles) and Sireniams (Fawcett, 1942).

2. At the microanatomical and histological levels, pachyostosis refers to both outward expansion of the compacta and the massiveness of the compacta relative to the cancellous bone and marrow spaces. This condition, which involves overall lowered porosity of bone tissue, is commonly but not necessarily related to anatomical pachyostosis, as described above. Marine (sirenian) ribs show an extremely thick, dense cortex of periosteal bone tissue, and in some cases the ribs may even lack a marrow cavity. The degree of mineralization per unit volume of tissue can also be increased in pachyostotic bone. Fossil pachyostotic bones have been described also in paleoichthyological studies (Meunier and Gaudant, 1987).

The term pachyostosis may be retained in a general sense for application at both the anatomical and histological levels irrespective of the adaptive or pathological mechanisms or functions involved. Pachyostosis has sometimes been equated with osteosclerosis (see below).

d. Hyperostosis (also spelled ‘hyperostosis’). This term is generally used in a pathological context for abnormally high amounts of primary bone deposition. In this case, the extra bone is not necessarily dense or compact, but it may consist of numerous fine cancellous trabeculae.

The numerous patterns of hyperostosis vary in their origin and pathological significance. For descriptive purposes at the level of gross morphology, hyperostosis may be simply understood as an increase in overall bone volume. However, in most cases it is used to describe specific pathological conditions, such as acetabular hyperostosis. Pachycephalosaurid dinosaurs and diberocephalid mammal-like reptiles show peculiar forms of hyperostosis in several skull bones, possibly as adaptations for ramming.

Spectacular examples of hyperostosis have been described in some living and fossil tetrapods (e.g. Carangidae, Sciaenidae, etc.) where typical flat bones (elephant, suprachephalic crests, etc.) or cylindrical bones (ribs, fin rays, etc.) become thick, swollen and more or less rounded (Ferstine, 1968; Desse et al., 1981). In the Teleostei, hyperostosis affects only the acellular bones of marine species (Desse et al., 1981). In fish the convergence of hydrostatic factors related to growth and specific environmental factors may explain these developments.

e. Osteopenosis: Osteoporosis refers to brittle and possibly hypermineralized bone with generally late development of marrow cavities and numerous remnants of calcified cartilages. This pathological condition may result from several distinct autosomal mutations, observed in rats, mice, rabbits and man. Osteoporosis is also known as marble bone and Albers-Schonberg disease. In most cases, the osteoclasts seem to have an abnormal function, resulting in more or less severe imbalances between bone deposition and resorption (Johnson, 1986). However, the physiological changes in these osteoclasts and osteoblasts may differ extensively from one mutation to another. Hence, osteoporosis is best understood as a general term which refers to the results of these abnormal conditions. In birds, hyperostosis produced by a retrovirus of the leucine sarcoma group has been called osteoporosis gallinarum. Although used as an experimental model for the study of hyperostosis, this condition has little to do with human osteoporosis (Dix et al., 1985).

f. Osteoscleroticus: This term refers to pathological endoskeletal bones in which the normal sequence of endochondral ossification is incomplete, resulting in large amounts of calcified cartilage retained in metaphyses and sometimes in the diaphysis. According to some authors, this situation is akin to osteoporosis and can be induced by a mutation, e.g., on chromosome 19 in the mouse (Johnson, 1986). On the other hand, it is interesting that osteosclerosis is commonly associated with pachyostosis in the endoskeleton of several non related tetrapods which share a secondary adaptation to aquatic life. This was recognized long ago by Nopca (1934) and Ricqlès (1975b). Rather than being pathological, such skeletal specializations can be understood as adaptations reached through heterochronic developmental processes.
B. Processes

1. Ossification. Ossification includes all processes resulting in bone formation. Normal ossification involves deposition of new bone on free surfaces rather than expansion from within already deposited bone. The prevailing process includes two stages:
   a. Matrix (collagenous) synthesis and extrusion by osteoblasts. This involves the self-assembly of fibrils into fibers or fiber bundles outside the cells, and the entrapment of these cells in the new matrix to become osteocytes.
   b. Progressive mineralization of the osteoid (the newly produced matrix) by hydroxyapatite, according to the spatial organization of fibrils.

Ossification can occur either directly in a specialized membrane (as in dermal bones and periosteum) or at the surface of the remains of "director trabeculae" in calcified cartilage (in endochondral ossification). A special variety of ossification includes hydroxyapatite deposition in preexisting dense fibrous tissues which are thereby changed into bone (i.e., metaplastic; see Beresford, 1981).

2. Parachondral ossification (Figure 8C). In parachondral ossification, bone forms directly within the connective tissue.

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Fig. 8. Bone organization and growth patterns. A: General organization of long bone shafts according to ecological adaptations; 1 = flying; 2 = land dwelling; 3 = water dwelling tetrapods. Regardless of the histological bone tissues involved, basic histological adaptations to environments with contrasting biomechanical constraints include variations in cortico-medullar proportions, contrast between compacta and spongiosa, and abruptness of the transition between the two regions. B: Generalized histological organization of a long bone shaft: a = primary periosteal lamellar bone laid down centrifugally; b = secondary osteon (Haversian system) formed of concentric lamellae laid down centripetally; c = longitudinal blood vessels in...
in the vicinity of a cartilage. The cartilaginous element appears to induce the shape of the bone, and the two elements are always separated by at least a layer of perichondrium and/or periosteum (Bujard, 1931; Blanc, 1933).

3. Perichondral ossification (Figure 8D). In perichondral ossification, bone appears in contact with an already formed cartilaginous element. The perichondrium stops making cartilage and it changes into periosteum. The cartilage then assumes the role of a mold upon which the osseous substance is deposited and thickens.

This process is especially conspicuous in the branchial arches of teleosts and along the diaphysis of the long bones in terrestrial vertebrates (Blanc, 1953). This term connotes a topographical relationship between bone and cartilage rather than an histological process. This is also true for parachondral ossification.

4. Dermal ossification. Dermal ossification leads to the formation of dermal bones, the genesis of which takes place only in the dermis (Agassiz, 1844; Owen, 1846; Kölliker, 1849). A dermal bone is sometimes tied to the ectodermal basement membrane by a surface coat of dentine and/or enameloid, or (in all tetrapods) it may lack such tissues. These bones form the superficial dermal skeleton (= dermoskeleton) of vertebrates. Dermal bones may be formed of lamellae, lamellar, cancellous, or combinations of these bone tissues. They are flat in shape and sometimes ornamented.

5. Membranous ossification. This is used by most authors as a synonym of dermal ossification. However, Patterson (1977) proposed a completely different definition as an ossification process of membrane bones which occurs deep in the mesoderm, below the striated musculature and without a preformed cartilaginous element. By this definition, membrane bones ossify with no ontogenic or phylogenetic connection with the ectoderm, and they belong to the deep endoskeleton (like cartilage bones). In the interest of eliminating confusion with the older, more generally accepted definition, Patterson's (1977) definition is best avoided.

6. Periosteal ossification (Figure 88). This results from activity of the periosteum at the outer surface of a bone, and it is characterized by a centrifugal mode of deposition (Gross, 1934).

7. Endosteal ossification. This results from activity of the endosteum on the inner surface of the cavities of a bone. This term is generally applied to ossification occurring after the resorption of a pre-existing bony tissue, and this endosteal secondary bone is separated from the primary bone by a cementing line. It is characterized by a centripetal mode of deposition (Flourens, 1845).

8. Endochondral ossification (Figure 9; French: ossification enchondrale). Endochondral ossification is the substitution of a preformed cartilaginous element with bony tissue, with the concomitant destruction of the cartilage model. Erosion of the cartilage is only partially complete so that many cartilaginous regions may persist within the osseous trabeculae, behind the front of erosion, at least in young bone.

This process characterizes the endochondral bones (= cartilage bones; French: os de remplacement; German: Ersatzknochen), which constitute the deep endoskeleton of vertebrates. This process is also met in fishes, where it occurs primarily in the vertebrae and skull (Blanc, 1953).

9. Metaplastic ossification. Metaplastic ossification occurs in two forms:

a. In pre-existing connective tissue. This involves "the progressive mineralization of the matrix and inclusion of cells without cell multiplication and cell hypertrophy" (Haines and Mohuiddin, 1968). Where there is already a dense connective tissue available, the cells can be included. Where mechanical requirements preclude the presence of a periosteum, a new bone is formed by 'metaplasia' (Haines and Mohuiddin, 1968), i.e., transformation of a pre-existing fibrous matrix of connective tissue directly into bone. This mode of metaplastic ossification may occur in the formation of osteoderms in reptiles (Levra-Culver and Zylberberg, 1980).

b. In cartilage. Chondro-osseous metaplasia is a direct transformation of cartilage into bone without concomitant destruction of the cartilage. The only non pathological example of this kind might be the "tussu mixte" of Stephann (1600). See also Breslau (1861) and 'chondroid bone' in Section IV-B, below.

10. Centrifugal and centripetal ossification (Figure 88). Centrifugal ossification is bone accretion occurring at the free external surface of a bone and proceeds outwards. Centripetal ossification proceeds inwards from the periphery of cavities within the bone.

Centrifugal bone is deposited by the periosteum around endoskeleton bones, or via periosteal-like cell layers in the skin dermis, as well as in fibrous sutures between dermal bones. Commonly, but not always, centrifugal bone tissues have a coarsely fibered matrix ('Faserknochen') of German authors.

Centripetal bone may be deposited by the endosteum around the narrow cavity, on trabeculae in endochondral ossification, or within resorption cavities of the deep cortex, according to the size and geometry of these spaces. This results in the compact bone of the 'inner fundamental system', secondary trabeculae of endosteal spongy bone, or secondary ostioles. This generally consists of finely fibered bone with a plywood-like structure, and it corresponds with the 'Schalenknochen', 'Markenknochen' or 'Quastenknochen' of German authors.

It is noteworthy that deposition seems to be much slower in centrifugal than in centripetal bone tissues.
Fig. 9. Endoskeletal bone growth and enchondral ossification. A: Six diagrammatic growth stages in a young mammalian long bone showing primary and secondary centers of ossification, cartilaginous growth plates, and articular cartilage. Periosteal bone is in black. From left to right: cm = early cartilaginous model of long bone; pb = cartilage deposition of periosteal bone; bv = blood vessel of the primary center of ossification; d = diaphysis; ep = epiphysis; m = metaphysis; 2c = secondary center of ossification; gp = cartilaginous growth plate; ce = cartilaginous epiphysis; eo = enchondral ossification in metaphysis; eo2o = enchondral ossification in secondary center; nc = nutrient vessel canals; mc = marrow cavity; ac = articular cartilage. B: Growth in length of a short bone. 1: cartilage proliferation occurring at both epiphyseal plates which have a circular shape. 2: A vertebral centrum lengthens (arrows) owing to enchondral ossification. C: Initial formation of the marrow cavity in the diaphysis of the femur of a young urodele amphibian, prior to enchondral ossification. From left to right: pb = periosteal bone (black or stippled, according to the staining reactions of the bone matrix); os = osteoblasts of the periosteum; ct = connective tissue in a longitudinal vascular canal in periosteal bone; oc = osteocyte; er = erythrocyte in a capillary; mc = medullary cavity; rf = radial vascular foramen with undifferentiated connective cells invading the marrow cavity; kl = remaining cartilaginous matrix forming Katschenko’s line around marrow cavity; ccl = chondroclast, giant, multinucleated cell destroying cartilage. Sources: A-C modified from Ricqlès (1965); B from Sinclair (1973).
11. Bone modeling (French: modélage osseux). Bone modeling is the acquisition and transformation of overall bone form and shapes by differential variation of local growth rates. The process of resorption-reconstruction (bone remodeling) is not necessary in this case, contrary to Enlow (1963) (Ricqlès, 1975a; Castanet, 1982; Frost, 1982, 1987). During morphogenesis, the bone may homothetically maintain its initial shape (isometric growth) or change its proportions and form (allometric growth). Beginning with an initial shape such as a cartilaginous model, conservation or transformation of shape results from the interplay of local osteogenetic rates in various directions (Ricqlès, 1975a; Castanet, 1982).

12. Drift (French: dérive osseuse). Drift of bones, as in the case of teeth, refers to the spatial transfer (generally lateral) and repositioning of parts of a bone, or the entire bone during growth (Enlow, 1963; Ricqlès, 1975a). Drift involves sequential relocation through bone resorption and bone reconstruction. Osseous drift can be detected at the histological level. Fluorochrome labels are valuable for detailed studies of osseous drift.

13. Skeletal growth patterns. Vertebrates exhibit numerous distinct growth patterns which are clearly linked to species-specific characteristics such as size and longevity, and which are of paramount adaptive significance. These factors are well emphasized by modern research on "demographic strategies". Because growth patterns are recorded by histological structures in the mineralized skeletons, it is obvious that knowledge of bone histology may help decipher growth pattern in fossils, and the converse is true for living animals. This is why definitions of skeletal growth patterns are in order here.

Continuous versus cyclical growth. Continuous growth connotes growth curves with progressively changing but always positive rates without cyclical changes. Conversely, cyclical growth connotes growth curves where rates change regularly, according to one or several cycles (daily, lunar, seasonal or yearly) with growth rate possibly reaching zero once every cycle.

Indefinite versus definite growth. Indefinite growth means that the animal maintains some growth as long as it lives. The growth rate commonly falls dramatically after reaching sexual maturity, but older animals will eventually become larger and larger (e.g. sharks, crocodiles). Definite (or finite) growth means that growth is possible only during an early phase of the animal’s life. Once adulthood is reached, growth rate falls to zero and stays there.

It is well known that among living vertebrates, endotherms and ectotherms have sharply contrasting growth patterns. Whereas ectotherms generally have cyclical, indefinite growth, the converse condition generally prevails among endotherms. These differences have well-known consequences regarding demographic and reproductive strategies. For instance, in a given population of ectotherms, reproduction may be performed at considerably different sizes, and age at first sexual maturity may be extremely variable. Also, larger (older) adults may improve their reproductive success. Among endotherms, on the contrary, variations in size and age of the attainment of adulthood appear to be much more narrowly and precisely constrained.

These generalizations have notable exceptions. For instance, many reptiles, in spite of their supposedly "indefinite growth", are characterized by a finite growth curve. Some anurans have even developed epiphyseal devices which prevent further growth (Francillon, 1981).

More importantly, the modern growth patterns generally linked to endo- or ectothermy in living forms may have been supplanted in fossil groups by growth patterns linked to different and now extinct strategies of metabolic and thermal physiology.

One may also mention that skeletal growth patterns may be related to species-specific properties of survival curves in ways that can introduce errors in skeletalchronological studies of population aging (Castanet, 1986-1987). Nevertheless, all the various conditions of growth can be recorded by growing bone tissues, and these conditions can potentially be deciphered through histological observations of bone among living and fossil vertebrates.

IV. The Histological Level.

Bone as a Tissue (Table 2)

This section covers the histological level of integration, namely how bones and other morphological elements of the skeleton are formed by specific tissues. Tissues are more or less complex associations of specialized cells and cell products which mutually cooperate and influence one another precisely defined patterns to fulfill specific functions. With regard to structure, the tissue level of integration is best studied with low to high magnifications of the compound microscope and with the SEM.

Bone as a tissue shows complex and highly variable structural patterns which need to be recognized and classified with regard to biological significance. Indeed, it may be more appropriate to speak of bone tissues from a descriptive, typological point of view, than from the perspective of 'bony tissue' as a whole. This is true for the sake of accuracy in description as well as because the tissue diversity of bone expresses to a large extent its various biological functions.

Most previous classifications of bone tissue structures have been based on single criteria. However, these monothetic classifications, although often straightforward and practical, were artificial, too purely descriptive, and far too simple to encompass the biological significance of bone tissue diversity. They were hardly appropriate bases for biologically meaningful natural classifications.

Also, confusion has been introduced by the fact that various authors have used bone histological terminology imprecisely, with 'classical' terms such as 'laminar bone', 'haversian bone', etc., used or understood in quite different ways.

Three distinct criteria have been commonly used as bases for the classification of bone tissues (Ricqlès, 1975a). One category of classifications relied exclusively on the organization of the bone matrix, which consists mainly of collagenous fibers. Hence we see such concepts as 'fibrous bone', 'woven bone', 'lamellar bone', 'shell bone', etc. A second category of classifications was based on patterns of bone vascularization. This gave rise to concepts such as 'non-vascular bone', 'plexiform bone', 'haversian bone', etc. The third and less clearly delineated basis for classification was based on ontogenetic patterns of bone tissue formation, which themselves encompass several valuable, but logically distinct and incongruous criteria. Diverse concepts such as 'centrifugal/centripetal bone', 'primary/secondary bone', 'periosteal/endochondral bone', etc., stemmed from this approach.

Progress in the knowledge of bone tissue diversity and its biological significance has led to the modern idea that all these criteria are valid and useful at least to some extent, and
TABLE 2. Classification scheme for bone tissue.

I. Bone matrices.
   A. Periosteal or superficial bone matrices.
      1. Fibrous or woven matrix of periosteal origin.
      2. Lamellar or parallel-fibred matrix of periosteal origin.
   B. Osteonal or internal bone matrices.
      1. Primary lamellar or parallel-fibred matrix of internal origin.
      2. Secondary lamellar or parallel-fibred matrix of internal origin.

II. Bone tissues.
   A. Compact bone tissues.
      1. Primary compact bone tissues.
         a. Lamellar\textsuperscript{1} non-vascular periosteal bone (lamellar-zonal tissue).
         b. Lamellar\textsuperscript{1} periosteal bone with simple vascular canals (lamellar-zonal tissue).
         c. Lamellar\textsuperscript{1} periosteal bone with primary osteons (lamellar-zonal tissue).
         d. Fibrous non-vascular periosteal bone\textsuperscript{2}.
         e. Fibrous periosteal bone with simple vascular canals\textsuperscript{2}.
         f. Fibrous periosteal bone with primary osteons: fibro-lamellar complex.
      2. Secondary compact bone tissues.
         a. Compacts with secondary osteons (Haversian bone tissue).
         b. Non-Haversian perimendular compacts.
   B. Cancellous bone tissues.
      1. Primary cancellous bone tissues.
         a. Spongiosa of dermal or periosteal origin\textsuperscript{2}.
         b. Primary endosteal spongiosa.
         c. Spongiosa of endochondral origin\textsuperscript{2}.
      2. Secondary cancellous bone tissues.
         a. Endosteal-endochondral spongiosa.
         b. Secondary cancellous deep cortex.
         c. Completely reconstructed spongiosa.

Notes: \(1\) Lamellar or parallel-fibred (pseudolamellar). \(2\) Tissue types occurring mainly during embryonic, fetal or larval stages. \(3\) Note that any kind of bone tissue consists of one or the combination of any of the four main bone matrices. Otherwise, description of the tissue type is readily obtained by taking into account: (a) its gross structure (compact vs. cancellous); (b) ontogeny (primary vs. secondary); (c) vascular pattern; (d) other particulars (cellular vs. acellular, special localization, etc.). The classification system is an open one, as it can generate any combination pertinent to the description of any actual tissue type. For that reason, categories of bone tissues given above under "Bone tissues" are only examples. For instance, in \(IIA\text{c}\), additional subcategories could be easily added by taking into account orientation(s) of the primary osteons.

that all must be combined and integrated into a single, biologically meaningful classification. As far as this typological classification would be natural, it could also be used as a framework for interpreting the overall functional significance of bone tissues (Ricqlès, 1975a).

These considerations provide the rationale for the seven separate subsets of definitions provided below. Subset A deals with the organization of the fibrillar (collagenous) matrix of bone. This widely used criterion deals, to a large extent, with a level of integration somewhat subordinate to the tissue level. Consequently, it can hardly be used alone to classify bone tissues. Indeed, the distinct bone matrices (woven, lamellar, parallel-fibred etc.), sometimes associate in various ways to give rise to actual tissue patterns. Subset B defines vascular patterns. Each vascular category can be variably associated with fibrillar matrices, each combination giving rise to specific bone tissue patterns. Subsets C and D take into account important aspects of bone ontogeny, such as bone remodeling and cyclic growth. Data from subsets A-D are combined in Subset E, where some distinct and important compact bone tissue patterns are recognized and defined. Note that all the known categories of bone tissue are not listed in subset 5 and need not be, because they can be readily generated from new combinations of the basic tissue components already recognized.

Table 2 summarizes some of the major categories of compact bone tissue and exploits the generating principles allowing the system to encompass new cases: the classification scheme remains an open one. Note that compact primary bone tissues can be subdivided into two broad categories defined primarily by the organization of the fibrillar matrix as lamellar-zonal (IIA\text{a-c}) or fibro-lamellar (IIA\text{d-f}). In the first case, bone tissue matrix of periosteal origin is mainly parallel-fibred or truly lamellar; vascularization, on the whole, is generally scattered and rarely very dense, and sometimes may even be lacking. Evidence of cyclical growth (annuli and zones) is extensive. In fibro-lamellar compact bone, the bone tissue matrix represents a complex of 1) a woven or fibrous, cancellous matrix of periosteal origin, and 2) a lamellar matrix,
Fig. 10. Patterns of deposition of bone tissue components. Diagrammatic cross section of a limb bone diaphysis, showing part of the cortex and cancellous medulla. 1: Primary compact cortical bone of periosteal origin, centrifugal deposition, circumferential (or tangential) relationships to longitudinal vascular canals; this bone forms the 'fundamental external system'. 2: Primary compact bone of endosteal origin, centripetal deposition, circumferential (or tangential) relationships to longitudinal vascular canals. 3: Cortical bone of primary osteons, centripetal deposition, circular relationship to vascular canals; 4: Medullary bone of 'primary' endosteal osteons, centripetal deposition, circular relationship to vascular canal. 5: Cortical bone of a secondary osteon, centripetal deposition around a vascular canal. 6: Medullar bone of a secondary endosteal osteon, centripetal deposition around a vascular canal. 7: Cancellous bone of the secondary endosteal spongiosa, centripetal deposition at random around medullar spaces. 8: Cancellous bone remains of the early "primary" endosteoo-endochondral spongiosa. 9: Secondary endosteal medullary bone, centripetal deposition, circumferential (or tangential) relationships to vascular or medullar spaces. Legend: cvps = simple primary vascular canals; Lci = reversal cementing lines; LH = erosion bays in the cortex or in the endosteal margin (periosteal region); opr = primary osteon; SH = Haversian system sensu stricto or secondary osteon. Source: From Ricqué (1975a).

cenpressally deposited, forming the primary osteons which ultimately fill the space originally empty in the woven periosteal matrix. Vascularization is therefore plentiful, although variable, and evidence of growth cycles is generally rare. Within these two general categories of primary compact bone tissues, vascularization can be used to sort out numerous well delineated typological categories.

One generalization of functional significance which emerges from this comparative classification of primary compact bone tissues is the relationship between tissue typology and rate of bone deposition. Subperiosteal deposition of densely vascularized 'fibro-lamellar' bone tissue is performed at rates considerably higher than most bone tissues lumped under the 'lamellar-zonal' pattern, as first recognized by Amprino (1947).

Others factors being equal, woven periosteal bone matrix is rapidly deposited, lamellar periosteal bone matrix is very slowly deposited, and parallel-fibered bone matrix is deposited at intermediate rates. Also, the denser the vascularity, the faster the deposition.

Finally, the two 'lumping' categories, viz 'fibro-lamellar' and 'lamellar zonal' should not be understood as mutually exclusive, radically distinct, or dichotomously contrasting. On the contrary they combine to form a continuum with all possible intermediate situations regarding matrix organization and vascularity.

Subset F deals with definitions of important categories of cell layers which are collectively responsible for the synthesis and deposition of bone extracellular matrices at the tissue level. Finally, Subset G deals with the mineralization of these matrices from a tissue point of view.

A. Bone Matrix Organization (Table 2)

1. Osteogenesis (bone deposition). This is the histocytopathological process of bone formation. It corresponds to: 1)
the secretion and spatial arrangement of collagenous and non-collagenous matrix by osteoblasts, and 2) the mineralization of this organic matrix, the mineral being mainly sals of calcium phosphate.

2. Woven-fibered bone matrix (Pritchard, 1956; Frost, 1960; Smith, 1960; Enlow, 1966; French: os à fibres enchevêtrées de Riquêlès, 1975a; German: Faserknochen). This consists of coarse and loosely packed collagen fibers of varying size distributed without any ordered spatial arrangement. Typically this bone matrix also contains randomly distributed osteocytes which are rather round, star-shaped and prolonged by many cytoplasmic processes running in canaluli. At lower magnifications, under a polarizing microscope, woven bone reveals general isotropy. This matrix is PAS positive, alcianophilic and strongly metachromatic with toluidin blue at pH 2.5. It may reach a high degree of mineralization. Woven bone is associated with rapid osteogenesis, which explains its poor spatial organization. Embryonic and most primary 'membrane' bones commonly consist of woven bone. It can be associated with a dense vascularization and peculiar 'growth lines' as in laminar bone tissue.

3. Parallel-fibered bone matrix (= PFB; thin fibbered bone; pseudo-lamellar bone; French: os à fibres parallèles; German: parallel faserige Knochen). Typically PFB consists of a large quantity of closely-packed collagen fibrils with the same general orientation, running approximately parallel to each other. Cells are flattened and more or less randomly distributed. PFB presents a mass anisotropy and consequently appears homogenously dark or light under polarized light, according to the orientation. In many respects (level of organization, histochemical properties, rate of deposition, included cells, relative degree of mineralization) PFB appears to be intermediate between typical woven and lamellar bone. Moreover, there are intermediate categories between PFB and both woven bone and lamellar bone, as expressed in the level of the fibrillar arrangement. It is important to note that transitions from PFB to woven bone or to lamellar bone are commonly present, even in the same section. These changes are functionally linked with the growth rate variations which they record.

4. Lamellar bone matrix (French: os lamellaire; German: Schalenknochen). Lamellar bone corresponds to a high level of spatial organization (Frost, 1960). It consists of successive thin layers called lamellae which typically appear alternately dark and light under crossed Nichols in polarized light. In each lamella the closely-packed collagen fibrils are mutually parallel but the direction changes from one lamella to the next. This arrangement corresponds to plywood structure sensu lato. From place to place some fibrils cross the lamella more or less transversely. Each lamella may contain some rows of flattened bone cells with few canaluli. Lamellar bone has generally lower mineralization than woven bone.

Until very recently, misinterpretations of the microstructure of lamellar bone lead to controversies about its fine structure and composition (Gehbardt, 1906; Weidenreich, 1930; Frank et al., 1955; Rouiller, 1956; Vincent, 1958; Ascenzi and Bonucci, 1968). However, Giraud-Guille (1988) seems to have provided the key to understanding the fine spatial organization of lamellar bone, and its varied optical characteristics are in agreement with those of many other biological plywood structures (e.g., crustacean cuticles, corneas of vertebrate eyes, isopedine; see below). Giraud-Guille distinguishes two main types of lamellar bone, but all possible transitions can exist: 1) orthogonal plywood where the angle between consecutive lamellae is 90 degrees, and 2) twisted plywood where fibrillar directions rotate from one lamella to the next with an angle different from 90 degrees. Its properties are then analogous to those of cholesteric liquid crystals which, under proper physical conditions, also spontaneously assemble in twisted systems. The microscopic features of lamellar bone, orthogonal or twisted, also differ if the plywood is flat (as in flat bones and scales) or cylindrical (as in secondary osteons). Optical features also change with the orientation of bone sections.

Functionally, the lamellar bone of primary corticals associates with a low rate of osteogenesis (e.g. less than 0.20 microns per day in reptile long bones). However this growth rate is generally higher for lamellar bone secondarily deposited, as in secondary osteons or endosteal bone at the periphery of medullar cavities.

5. Plywood-like structure (Figure 11). The word 'plywood' was used by Weiss and Ferris (1954) to describe an arrangement of collagen fibrils resembling the cross-ply of the wood layers in plywood. In the same layer the fibrils are mutually parallel but the directions are different in adjacent layers (Meunier and Géraudie, 1980). Plywood-like structure differs from typical lamellar bone matrix in that its collagenous fiber bundles can attain considerably larger sizes. Plywood-like structure has been described in lamellar bone and in the basal plate of elasmoid scales. The structure of the latter is quite spectacular, with thick layers (up to 30 microns) and large diameter collagen fibrils (30 to 190 nanometers). In contrast, lamellar bone has thinner layers and smaller diameter (20 to 50 nanometers) collagen fibrils (Meunier, 1984b). The basal plates of all elasmoid scales in living fishes (Dipnoi, Latimeria, Amia, and teleosts) have a plywood-like organization; similar organization exists also in the elasmoid scales of various fossils, in the Sarcopterygii (e.g., Holoptychius) and Actinopterygii.

In plywood-like structures mineralization may be delayed or completely lacking, as in Latimeria and Neurocrateri. Moreover, cells (elasmocytes) are sometimes incorporated in (Latimeria, Dipnoi, Amia, Osteoglossiformes, etc.) in some 'less evolved' fishes (Latimeria, Polypterus, Osteoglossiformes, Cypriniformes) vertical fibers (TC fibers, transverse fibers) cross the plywood layers.

Two main types of plywood-like structure have been defined: orthogonal (Meunier and Castanet, 1982) and twisted (Giraud et al., 1978a,b) (see 'lamellar bone tissue, E-9, in this section). The orthogonal type can be divided into true orthogonal and non-stabilized orthogonal (Meunier and Castanet, 1982; Meunier, 1987a). The latter two are differentiated by the more or less regular and stable pattern of successive plies.

The plywood-like structure of elasmoid scales probably evolved from the more or less complex dermal elements of rhomboid scales. The typical plywood of elasmoid scales constitutes isopedine, according to Meunier (1983, 1987a).

6. Isopedine. The basal plates of elasmoid scales in the Ostichthyines show various associations of collagen fibrils, elasmocytes and mineral, i.e. the same components as bone. However, whereas some scales have all three components, others may lack one or two of them, either elasmocytes and/or mineral. This situation conflicts with the current concept of bone tissue. Therefore Meunier (1983, 1984b) proposed using 'isopedine', a term coined by Pander (1856),
Fig. 11. Organization of isopedine and plywood-like structures in scales. A: External morphology of a scale, viewed from above, from *Latimeria chalumnae*. B: Vertical section through the scale in A, along the transect b-b', showing the basal plate (isopedin), outer layer, and odontodes. C: Detail of the inset in B, showing the successive layers of collagenous fibers. The large arrows on the left point to layers where the fibers are cut perpendicularly; note that this fibrous direction occurs in every seventh layer. D: Model of an oblique section through isopedin. Layers 1/2, 3/4, etc., form successive orthogonal piles and their orientation rotates progressively in the direction of the arrows. E: The separation of the two sets of layers in D, even and odd, allows one to observe the progressive rotation of fibrillary systems and the arched patterns appear. F: Reconstruction of the fibrillary directions in the successive layers of the scale basal plate according to the model in E. **Legend:** Ca = anterior region; Cc = external layer; Cp = posterior region; Cpb = elasmocyte; De = odontode; Fi = fiber; Pb = basal plate. **Sources:** A,B,C from Meunier and Géraudie (1980); D,E from Giraud *et al.* (1978a); F from Giraud *et al.* (1978b).

for the plywood-like tissues which constitute the basal plate of elasmoid scales.

Pander (1856) used 'isopedine' for the histological description of fossilized dermal elements which, some years later, were assigned to *Trematospis* (Agnatha) by Rohon (1893). Their basal plate consists of "numerous superimposed, parallel and horizontal layers with elongated cells", the orientation of the long axes of which "is influenced by the organization of the lamellae where they are inserted". Furthermore, Gross (1968) showed that the basal plate in *Dartmouthia*, an Osteostraci related to *Trematospis*, has a typical plywood-like structure with thick fibers. Following Goodrich (1907), numerous authors have used 'isopedin' to describe the basal plate of various rhomboid scales, particularly polypterid scales, showing simple osseous tissue that does not generally show characteristic plywood-like structure.

As suggested by Gross (1968), Giraud *et al.* (1978a,b) and Meunier (1984b, 1987a,b) it now appears necessary to restrict 'isopedin' to lamellar structures in the basal plates of
dermoskeletal formations that show typical plywood-like organization. This is compatible with the usage by Pander, who coined this term to simplify his paleohistological descriptions.

One can define several isopedins, all of which show more or less important specializations including the complex plywood-like organization, a lack of incorporated cells (= elastocytes), and regression or even lack of a mineral component. These evolutionary changes have appeared in various lineages of Osteichthyes through heterochronous convergence (Meunier, 1987a), and they are probably linked to a general reduction of dermal ossification since the Paleozoic, and to functional adaptations, especially those related to improved swimming (Burik, 1979).

7. TC fibers. This term, coined by Onozato and Watabe (1979), describes the 'sheet-like structure' which occurs in the basal plate of elasmoid scales in the Cyprinidae. TC fibers are thin collagen fibrils oriented perpendicular to the thick collagen fibrils forming plywood-like structure. Like the latter, they are synthesized by the elastoblasts lining the basal plate. TC fibers appear to be involved in the first stage of mineral deposition in the basal plate (Schönberger et al., 1979; Zylberberg and Nicolas, 1982).

8. Sharpey's fibers. Named by Kölliker (1889), Sharpey's fibers are the fibrillary attachment processes of such soft tissues as muscles, tendons, ligaments, dermis, and desmodonts within mineralized skeletal tissues. They consist of closely-packed collagen fibers (bundles) which insert more or less perpendicularly into the mass of collagen fibers in the anchoring bone. This organization corresponds with 'bundle bone' (French: os fascicule). Sharpey's fibers are mineralized, and this mineralization may extend beyond the level of the bone tissue surface (Boyle, 1972). These fibers are unknown in enchondral bone tissue (Stern, 1964; Bevelander and Nakahara, 1968; Boyle and Jones, 1968; Boyle, 1972; Baron, 1973; Jones and Boyle, 1974; Zylberberg and Meunier, 1981; Johnson, 1987).

9. Anchoring fibers (= anchoring bundles; French: fibres d'ancrage). These are merely Sharpey's fibers which anchor soft tissues such as the epidermis and dermis to a scale surface. They occur only in the posterior region of elasmoid scales. They are probably synthesized by the superficial scleroblasts after the external scale layer is deposited. Anchoring fibers have the same diameter as the fibrils of the external layer of the scale (30 to 40 nanometers). These fiber bundles extend from the external scale layer in young fishes or from the outer limiting layer in old specimens to the basal lamina of the epidermal-dermal junction. They progressively mineralize in the region where they are surrounded by the matrix of the outer limiting layer. Mineralized spherules, probably containing substances of epidermal origin, are involved in the mineralization process (Sire, 1988). See also Zylberberg and Meunier (1981) and Sire (1985b).

10. Pre-osseous layer (= osteoid, pre-bone; French: liséré préosseux). This is the newly formed and unmineralized organic matrix deposited by osteoblasts. Osteoid contains normal D-periodic collagen fibrils and non-collagenous proteins which possibly differ slightly in composition from those of the underlying mineralized bone. The existence of osteoid has been demonstrated by Lacroix (1954), Vincent (1955) and Ponlot (1960). Solochrome cyanine is one of the most useful dyes for detecting osteoid in histological sections (Matrajt and Hioco, 1966). Use of the term 'osteoid', as proposed by Kölliker (1859) for acellular bone in fishes, should now be avoided. See also 'acellular bone tissue', E-2, below in this section.
11. Frontier line (French: ligne frontière). See ‘mineralization front’ below, G-1, this section.

12. Cementing lines (French: lignes cimentantes). Cementing lines may represent two kinds of thin (1-2 micron) discontinuity layers encountered in bone tissues:
   a. Resorption lines (= reversal lines, remodeling lines). These cementing lines typically appear irregular and scalloped because they represent deposition on previously resorbed bone surfaces, on which they lie unconformably. Resorption lines permit the identification of bone remodeling features, thereby making it possible to distinguish primary and secondary bone, secondary osteons, etc.
   b. Resting lines (= rest lines) occur only on unresorbed bone surfaces when osteogenesis resumes after a temporary cessation. Unlike resorption lines, resting lines appear more or less smooth and unscalloped. Cementing lines correspond to regions of mechanical weakness. When bones are subjected to strain and/or fossilization, bone splits first appear along these discontinuities. Cementing lines are strongly hematoxylinophilic. In unstained bone, they appear as bright and refringent lines under ordinary and polarizing light respectively. Although some disagreement still exists regarding structure and composition of cementing lines, it is clear that they are not completely lacking in collagen fibers. ‘Ground substance’, probably with high sulfur content, may be more concentrated in cementing lines than in other parts of bone. However, some cementing lines attain a degree of mineralization greater than the most highly mineralized parts of bone, such as woven fibered bone (Maturaj et al., 1964; Castanet, 1979, 1981; Buffrénil, 1982).

B. Vascularization (Figures 10 and 12)

1. Vascular and avascular bone. Following Enlow and Brown (1956, 1957, 1958), Enlow (1963), and Ricqlès (1975a) we can distinguish four major categories of bone vascularization:

<table>
<thead>
<tr>
<th>TABLE 3. Categories of bone vascularization.</th>
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<tbody>
<tr>
<td>1. Non-vascular bone tissues.</td>
</tr>
<tr>
<td>2. Bone tissues with primary vascular canals (notably primary osteons) orientated in one direction. The orientation of the canals is defined relative to the overall bone shape and geometry.</td>
</tr>
<tr>
<td>a. Longitudinal canals: the canals are mutually parallel but may be homogeneously dispersed in the thickness of the compact bone, or more or less regularly arranged in circular, radial or oblique bundles.</td>
</tr>
<tr>
<td>i. Longitudinal canals organized in circular bundles.</td>
</tr>
<tr>
<td>ii. Longitudinal canals organized in radial bundles.</td>
</tr>
<tr>
<td>iii. Longitudinal canals organized in oblique bundles.</td>
</tr>
<tr>
<td>b. Circular vascular canals.</td>
</tr>
<tr>
<td>c. Radial vascular canals.</td>
</tr>
<tr>
<td>d. Oblique vascular canals.</td>
</tr>
<tr>
<td>3. Bone tissues with primary vascular canals oriented in more than one direction.</td>
</tr>
<tr>
<td>c. Reticular vascularization. Regularly but obliquely oriented vascular canals.</td>
</tr>
</tbody>
</table>

Histological definitions of bone tissue categories should take in account not only their vascularization pattern, but also and simultaneously their fibrillar organization.

2. Simple primary vascular canal. This is a thin vascular canal without surrounding bone lamellae (Enlow, 1963; Ricqlès, 1975a). The vessel is merely enclosed within the mineralizing matrix of new appositional bone, without further complications.

3. Primary haversion cavities (= primary Haversion spaces; French: espaces haverviens primaires, ou primitifs). These are generally large holes or cavities which are initially left open in young, often fast-growing, bone. The cavities range from about 50 to 250 microns in diameter and they may or may not be interconnected, forming a more or less dense and complex network. They are initially filled by numerous fibroblasts, soft connective tissues and blood vessels. They are lined by newly formed bone, generally, but not necessarily, with a woven or fibrous matrix. The latter bone tissue is deposited in a fine cancellous pattern. Subsequently, the spaces are progressively filled with centripetal bone with a lamellar or parallel fibered matrix, laid down by the osteoblasts which differentiate in situ. This centripetal deposition forms the primary osteons. The centripetal deposition does not completely fill the primary cavities, however, because a primary vascular canal (containing one or two blood vessels) generally remains open in the axis of the osteon, as part of the intrinsic bone vascular network.

The primitive Haversian spaces are associated with the formation of the primary osteons but the latter are not the Haversian system proper. The ‘Haversian spaces’ are perhaps better termed the ‘early or primitive cavities (spaces) of primary (often finely cancellous) bone’, the term ‘primary’ being more meaningful than ‘Haversian’.

4. Primary osteon (French: ostéone primaire, ostéone d’addition). This is a vascular canal surrounded by concentric bone lamellae which are centripetally deposited without resorption at the previous periphery of the canal (Gross, 1934; Ricqlès, 1975a). Primary osteons occur in primary, appositional bone, and their formation requires no resorption of previously deposited bone tissue, unlike secondary osteons or Haversian systems sensu stricto.

5. Secondary osteon (= Haversian system; French: ostéone secondaire). These are erosional lacunae initiated from a vascular canal and secondarily filled by centripetally-deposited, concentric bone lamellae. Secondary osteons are always delimited from the surrounding bone by a cementing line of resorption (Biedermann, 1913; Gross, 1934). A vascular canal in the center of a secondary osteon is called an
Haversian canal. Its diameter varies according with the amount of secondary centripetal lamellar bone deposition (the wider the canal, the 'younger' it is). See Ponlot (1960), Matrajt et al. (1964) and Ricqlès (1975a).

6. Haversian systems (French: systèmes de Havers, ostéones de substitution, ostéones de remplacement). This famous term in bone histology has been overused and abused to refer to nearly any kind of concentric structure in bone. This has unfortunately led to great confusion. Following Gross (1934) and Amprino and Godina (1947), most specialists now agree that the Haversian system proper is synonymous with secondary osteons (Enlow, 1962; Ricqlès, 1975a) (see no. 5, above).

Complete bone shafts in transverse sections have been equated with a single giant Haversian system. However, this is a misleading comparison because primary periosteal deposition is centripetal whereas osteonal (secondary, Haversian) deposition is centripetal. A more meaningful comparison is between Haversian systems and bone deposited centripetally by the endosteum, either regularly around the medullar cavity (the internal fundamental system) or irregularly as trabeculae of the secondary spongiosa.

Indeed, all transitions may be observed ('endostal osteons'), between coarse cancellous endosteal tissue of the medullar region, formed by scattered secondary trabeculae of endosteal bone, and typical Haversian systems in the deep cortex.

Terms such as 'proto-Haversian', 'pseudo-Haversian osteons or systems', etc., should be discarded because they have been variously used in confusing and contradictory ways both ontogenetically and phylogenetically, for both bone and dentine.

7. Primary and secondary bone (French: os primaire et os secondaire). According to the general definitions used in comparative histology (Gross, 1934; Amprino and Bairati, 1936; Amprino and Godina, 1947; Smith, 1960; Ricqlès, 1975a), primary bone corresponds to bone deposited where antecedent bone tissue does not exist; and secondary bone corresponds to bone deposited in areas where an antecedent bone tissue has been resorbed. Secondary bone is bone of substitution (Ricqlès, 1975a) and it is typically associated with cementing lines of resorption.

It may be useful to recall that the concept of primary and secondary bone involves neither the fine structure of bone tissue nor a general temporal, ontogenic or phylogenetic sequence (Lacroix, 1970). It is also important to note that the concept of primary and secondary bone (of which primary and secondary osteons are a particular case) has only a relative morphological significance. We have recently shown (Castanet and Ricqlès, 1986-1987) that if secondary bone can be locally deposited as primary bone, both being clearly distinguished from each other by typical cementing lines of resorption, they can nevertheless show locally perfect fibrillar structural continuity. Consequently the concept of primary and secondary bone (or osteon) remains clear only in the context of very local histogenetic circumstances in space and time.

8. Haversian tissue (Figure 15B; French: tissu Haversien). In compact bone, the secondary osteons (= Haversian systems) can be widely separate or very close together. In the latter case, when successive generations of closely packed secondary osteons intersect each other (= ostéones de remplacement ou de substitution', Ponlot, 1960; Lacroix, 1970) they produce a pattern called dense Haversian tissue.

In the rostrum of the sword fish, Xiphias gladius, a peculiar Haversian tissue consist of complex polyosteenal units of acellular bone (Castanet and Ricqlès, 1986-1987). The Haversian canals are the vascular canals in the center of each secondary osteon.

In dense Haversian bone tissue, several generations of Haversian systems may be superimposed in situ. Cross-cutting relationships may be used to decipher which local system is relatively younger. Successive secondary osteons in the tissue may be termed secondary osteons (or Haversian systems) of first, second, third generations, etc.

Intersitial systems (or lamellae) are the remnants of former secondary osteons partially eroded away. Extensive lateral drift of reconstructed osteons are commonly observed in dense Haversian tissue.

In loose Haversian bone tissue, or in compact bone which has only recently started to become replaced by secondary osteons, large remnants of the previous (primary) bone can be left and the initial tissue type readily recognized. All transitions may be found in compact bone tissues in the amount of Haversian replacement.

The rate of bone substitution may vary widely and can be checked experimentally by bone labelling in vivo (Epker and Frost, 1965). In fossil material, indirect evidence on this problem may be also gathered by the amount of completely rebuilt osteons relative to the amount of partially rebuilt ones, or the amount of active, open erosion spaces to completed secondary osteons, etc.

9. Volkman's canals. The term Volkman's canals is commonly used for all radial vascular canals encountered in compact bone, periosteal or endosteal (Jaffe, 1929; Petersen, 1930). However, as summarized by Ricqlès (1975a), this term should be reserved only for radial primary vascular canals in compact bone. The original definition was addressed to pathological structures and is seldom used today.

C. Bone Remodeling and Resorption (Figure 10).

1. Bone remodeling (French: remanement osseux). This corresponds to the normal and more or less continuous reshaping of a bone, mainly related to morphogenesis during early life (Enlow, 1963), but also related to mechanical constraints and physiological demands later in life (Amprino, 1948, 1967; Dhern, 1967). Bone remodeling involves the dual processes of bone resorption and bone redeposition (see below). Consequently, bone remodeling is always linked to the presence of cementing lines of resorption and vice versa. Bone remodeling takes place inside and at the periphery of bones. It can affect primary as well as secondary bone tissues, compacta as well as spongiosa. Bone remodeling is associated with the phenomena of bone relaxation and bone drift (Enlow, 1963).

2. Bone resorption. Bone resorption may involve two processes: a) Osteoclasia, the erosion of bone surfaces by osteoclasts, produces bone surfaces that appear scalloped (Hawship's lacunae) and notched. b) Periosteocytic osteolytic bone resorption by osteocytes at their own periphery. Conventionally the term osteolysis deals only with this aspect of bone resorption.

D. Growth Marks (Figure 13).

Also known as incremental growth layers, growth rings, and in French as 'marques de croissance squelettique',
Fig. 13. Bone growth marks. Cyclical apposition of new primary bone is recorded by the tissue structure according to the local pattern of growth, and may be used to assess the individual's age, growth rate, etc. A: Diagrammatic sections showing various relationships of growth marks to different histological types: 1 = growth marks in a poorly vascularized compact lamellar-zonal periosteal bone; 2 = growth marks in a rather densely vascularized lamellar-zonal cortex (vascular canals are localized in the zones, which can consist, in part, of poorly organized, woven bone); 3 = growth marks in avascular bone tissue; in the region of active growth, zones are formed of avascular fibrous bone and annuli of lamellar bone; in regions of slower growth (right) the zones are formed of lamellar bone and the annuli are reduced to a simple line of arrested growth (L.A.G.). B: Diagrammatic synthesis of the growth marks recorded in a bone with distinct growth regions (parts 1 and 2). Part 1: a region actively expanding in one direction with anisodiametric growth as in long bone apophyses or flat bones; zones consist of fibrous or woven bone and annuli of lamellar bone, as in A3 (on left); annuli are generally marked by a line of arrested growth (L.A.C.) and are commonly reduced to a L.A.C. Part 2: a region with isodiametric growth, a common condition in long bone shafts as viewed in cross-section; the record of cyclical growth is similar to that shown in Figure 13A1 or 13A2, according to local growth dynamics. Local morphogenetic dynamics control the histological expression of growth marks. Legend: rI = resorption line; 20d = secondary deposition of new bone over a resorption line; rc = cavity of internal resorption; mc = medullary cavity with the position of virtual early annuli partially eroded away. Sources: A from Ricqlès (1975a); B from Castanet et al. (1977).

growth marks represent any variation in growth rate recorded at the morphological or histological levels in any sclerified tissue (e.g., chitinous insect cuticles, keratinous turtle scales, sheep horns, mineralized mollusc shells, echinoderm endoskeletons, fish otoliths and scales, and vertebrate bones and teeth).

Growth marks generally show a definite periodicity in accordance with the growth rhythms of the animal. Thus they appear as successive layers producing stratified patterns at the surface or within the sclerified elements. Growth marks are useful for understanding growth events affecting various organs or whole organisms throughout their lives. For instance, they are very useful for individual age determination and for studies of morphogenesis and growth rate (Klevezal and Kleinenberg, 1967; Grue and Jensen, 1979; Perrin and Myrick, 1980; Klevezal, 1988).

In vertebrate mineralized tissues, especially bone, growth marks may constitute zones, annuli, and rest lines (see...
below). Generally one zone plus one annulus and/or one rest line corresponds to a yearly cycle. However, one pair of growth marks may alternatively represent a double annual cycle (Caetano et al., 1985; Lecomte et al., 1985) or even a daily cycle. The study of growth marks is called *skeletochronology* or, more commonly, *sclerochronology*. *Sclalmetry* and *otolithometry* are more specialized approaches dealing with growth marks in fish scales and otoliths, respectively.

1. **Zones**. A zone corresponds to a period of high growth rate (not necessarily annual) and it is therefore thicker than an annulus or a rest line (Peabody, 1958, 1961; Warren, 1963). Zones in bone consist of woven bone or parallel-fibered bone with relatively abundant and roundish osteocytes and sometimes with a dense network of canalicular. They appear relatively dark under transmitted light, but bright in reflected light. Zones are the most opaque of the growth marks. If bone is vascularized, vascular canals are more abundant or exclusively present in the zones.

2. **Annuli**. These are narrower than zones and they correspond to periods of slow growth. In bone, they consist of parallel-fibered bone or, more commonly, of lamellar bone. They are less abundant than growth annuli that they may be slightly more mineralized. Inside growth annuli the cells are flattened, with poor canalicular development, and sometimes even lacking.

3. **Rest lines** ("lines of arrested growth" of Zug et al., 1986; French: lignes d’arrêt de croissance). Rest lines are one of the two main categories of cementing lines (see above). They may occur alone or within an annulus, and they correspond with temporary but complete cessations of growth. Lines of arrested growth, or annuli when present, are preferentially used for individual age estimations. Rest lines can be deposited in primary as well as in secondary bone, e.g., in secondary osteons (Lacroix, 1970), but in the latter case their value as chronological indicators is very doubtful (see also Castanet, 1985; Castanet and Naulleau, 1985, and Castanet, 1986-1987).

### E. Typological Classification of Bone Tissue (Table 2)

1. **Cellular bone tissue**. Cellular bone is any kind of bone tissue containing bone cells (osteocytes) enclosed in (periosteocytic) bone lacunae. The word "osteoplast" for bone cell lacunae was used in the older literature, but this is seldom used today. Typically, bone cells remain alive and physiologically active, communicating with each other by means of fine cytoplasmic extensions housed in minute bone canaliculi. The volume, shape, abundance and canalicular development of osteocytes can vary greatly from one bone tissue to another. Such differences commonly reflect rate of deposition and/or the metabolic activity of the bone tissue. The orientation of osteocytes also varies according to the spatial organization of the neighboring bone matrix.

Osteocytes are 'mature bone cells' resulting from the entrapment of former osteoblasts within the mineralizing bone matrix. However, some osteocytes do not derive from typical osteoblasts of the periosteum or endosteum. This is the case for bone cells within the densely fibrous mineralizing tissues forming the insertion sites of ligaments and tendons.

2. **Acellular bone tissue** (= anosteocytic bone; French: os acellulaire). As the name indicates, acellular bone tissue is devoid of osteocytes. More precisely, acellular bone never contains complete osteocytes in typical perilosteocytic lacunae, but, at most, fine cytoplasmic extensions of bone cells housed in long minute canaliciuli. When acellular bone contains fine cytoplasmic extensions of bone cells, most of the cytoplasm, which remains close to the bone cell nuclei, recesses before the mineralizing extracellular matrix. This gives rise to a bone tissue which looks superficially like osteodentine. This similarity led to confusion in the early literature because acellular bone tissues in higher teleost fishes (where this condition is common) were mistakenly described as dentine (Stephan, 1900; Blanc, 1953; Moss, 1961, 1965).

Alternatively, acellular bone may entrap young bone cells (osteoblasts) within the bone matrix, but these cells ultimately shrink and die before mineralization takes place.

Note that: a) acellular bone may be vascular, although acellular avascular bone also occurs; b) acellular bone may experience erosion and secondary reconstruction, hence acellular secondary osteons may occur in acellular bone; c) if vascularization is lacking, acellular bone generally shows well developed canalicular systems (hence it is superficially dentine-like as explained above); but conversely, well-vascularized acellular bone may not contain cell-canalicul systems, or these systems may be very short and restricted to small regions close to the lumen of vascular canals.

Earlier descriptions referred to acellular bone as 'osteoid'. However this term should not be applied to acellular bone because it now designates that part of newly deposited bone matrix which is not yet mineralized.

Although acellular bone occur in various groups of vertebrates, it is by far most common among advanced teleosts (highly evolved bony fishes), as first recognized by Kölliker (1859). Hence, at least among bony fishes, acellular bone represents an apomorphic (advanced, or specialized) rather than a plesiomorphic (primitive, or generalized) condition (Weiss and Watabe, 1979; Meunier, 1987a).

3. **Aspidin**. Aspidin is a variety of acellular bone which occurs in the dermal skeleton of the earliest vertebrates, the jawless heterostraceans (or *paraspidomorphs sensu lato*) of the Early to Middle Paleozoic. Aspidin, as a tissue, may show various patterns, e.g. cancellous or lamellar, as in the middle and basal regions of dermal plates, respectively. Aspidin is generally covered by superficial odontodes, the pulp cavities of which communicate with the cancellous spaces of the aspidin. Although most aspidin appears to be a primary tissue, there is some evidence for erosion and reconstruction (secondary deposition) and hence aspidin may resemble cellular bone in this respect.

Because aspidin is among the geologically oldest (if not the oldest) vertebrate hard tissues, many theories have been advocated that aspidin is the phylogenetic predecessor of cellular bone. This hypothesis has prompted considerable discussion *pro* and *con* (Halstead-Tarlo, 1963; Ørvig, 1965; Halstead, 1973; Ricqlès, 1979; Meunier, 1983).

On the other hand, some authors have denied the acellularity of aspidin, interpreting some mineral-infilled minute spaces as bone cell lacunae. The name 'aspidinocytes' has even been coined for these presumed cells. However this interpretation is not generally accepted today by most specialists.

4. **Aspidone**. Aspidone refers to circular centripetal deposition of matrix around a vascular space in aspidin. Aspidone is, for all intents and purposes, analogous to the concept of primary osteons within cellular bone.
5. *Avascular bone tissue.* This is compact bone tissue which is completely devoid of an intrinsic vascular network. The term refers to compact bone because the various spaces in cancellous or spongy bone commonly contain numerous blood vessels. Primary avascular bone contains neither simple primary vascular canals nor primary osteons. It is seldom reconstructed by osteoclastic erosion and hence it generally lacks secondary osteons. The description of compact avascular bone may be based on considerations of the extracellular matrix (woven-fibered, parallel-fibered, lamellar) and included osteocytes, when present. These osteocytes commonly develop extensive systems of canaliculi which may in part substitute physiologically for the lack of vascular canals. These canaliculi are actively involved in bone-body fluid mineral exchanges, as evidenced by periosteocyte osteolysis and perilacunar demineralization halos (Alcobendas and Baud, 1988).

Avascular bone forms most of the compact bone tissue of living lizards and snakes. It shows a general "lamellar-zonal pattern" of organization, with parallel-fibered and/or lamellar bone matrix forming the bulk of the tissue, and often showing extensive evidence of cyclical growth (annuli and zones).

6. *Vascular bone tissue* (Figures 12 and 14). This refers to any bone tissue containing an intrinsic network of blood vessels. Appositional (primary) compact bone tissue can be divided into subcategories according to the nature (simple primary canals; primary osteons), number, and orientations (longitudinal, radial, etc.) of its vascular canals. Similarly, secondary (remodeled) compact bone can be divided into subcategories taking into account the nature, number, and orientation of both the primary and secondary (Haversian) osteons in the tissue. However, as vascular canals in primary compact bone are also associated with various categories of bone matrices (see below), a precise typological description of compact vascular bone tissue should combine information of both vascularization and fibrillar organization.

In most vertebrates, living and fossil, primary compact bone tissues in both the dermoskeleton and endoskeleton pertain to one or another subcategory of vascular cellular bone (Enlow and Brown, 1956-1958).

7. **Woven-fibered bone tissue** (= fibrous bone; French: os fibreux, os à fibres enchevêtrées; German: Faserknochen, geflechtartigen Knochen). This is any bone tissue in which a fibrous or woven bone matrix (see above) forms the bulk of the tissue. A woven matrix in compact bone is generally deposited by the periosteum or by the fibrous sutures between dermal bones. It is very rarely, if ever, endosteal in origin. Bone tissues exclusively formed of a woven matrix are generally found in embryos and in very fast growing young individuals, especially among higher vertebrates. The bone, rather than forming a compacta, is generally laid down

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*Fig. 14. Typological classification of compact primary bone tissues. A: Lamellar-zonal tissue with primary longitudinal osteons and radial simple vascular canals. B: Fibro-lamellar tissue with primary longitudinal osteons. C: Fibro-lamellar tissue with primary osteons oriented in radial rows. D: Fibro-lamellar tissue with primary osteons oriented in circular rows. E: Lamellar bone tissue (fibro-lamellar tissue with primary osteons in circular rows and forming circular canals). F: Plexiform bone tissue (as in E, but with the addition of radial vascular canals). G: Radiating fibro-lamellar bone tissue (as in F, but with prevalent radial primary osteons and reduced circular primary osteons). H: Reticular fibro-lamellar bone tissue (all primary osteons have an oblique orientation and anastomose irregularly). Note: Dotted pattern indicates fibrous or woven periosteal bone. All variations of the 'fibro-lamellar complex' (B-H) may be locally transitional from one type to another; the 'lamellar' part of this complex is provided by the numerous primary osteons which can account for more than half the total amount of bone tissue. Source: from Ricqlès (1975a).*
as a fine cancellous tissue (Amprino and Godina, 1947). This rapidly deposited bone 'scaffolding' is rarely a permanent component of the skeleton. In most circumstances, this bone tissue is either eroded away during later growth or it is completed by centripetal deposition of fine lamellar bone matrix forming primary osteons in the vascular spaces (or primitive Haversian spaces).

Woven bone tissue can contain more or less extensive amounts of extrinsic (Sharpey's) fibers and it seldom forms a significant part of the spongosia in the medullar regions of the bone.

Where woven or fibrous bone matrix is associated with lamellar matrix of primary osteons, the structure is called 'fibro-lamellar bone tissue' (Ricklès, 1975a). This general term 'lumps' together these important and commonly associated typological categories of primary bone compact tissues (see below).

8. Parallel-fibered bone tissue (= pseudo-lamellar bone; French: tissu osseux à fibres parallèles; German: parallel faseriger Knochen). This is any bone tissue characterized by a matrix consisting primarily of mutually parallel collagenous fibers. In compact primary bone tissues, parallel fibred bone is most commonly cellular and vascularized by simple primary vascular canals and/or primary osteons. The orientation, number and possible anastomoses of the vascular canals are used to define subtypes of these tissues. Compact bone tissue of periosteal origin consisting of parallel-fibered bone matrix is sometimes avascular; vascularization, when present, is generally not very dense. This bone tissue is often organized in superimposed 'zones', each forming the bulk of the bone laid down during the stage of fast deposition in a yearly growth cycle. It may contain extrinsic (Sharpey's) fibers if it has a dermal, sural or periosteal origin. In the internal region of bones consisting of coarse cancellous tissues, extensive fine, parallel-fibred bone tissue is laid down by the endosteum, and this can form an important part of the bone trabeculae lining the medullary sinuses. Generally this bone experiences extensive cycles of erosion and reconstruction, so it is largely a secondary bone tissue. All intermediate conditions exist between the typical secondary trabeculae of the spongosia and the more or less large, well-defined secondary osteons in the endosteal margin (the transitional region between deep cortical bone and the medullary spongosia). Indeed both can be built of fine parallel-fibred bone laid down by the endosteum.

In some groups, even the fibrous matrix of primary osteons may be parallel-fibred (bony fishes, birds), a condition also seen in ossified tendons.

9. Lamellar bone tissue (Figure 14; surface bone pro parte; French: tissu osseux lamellaire; German: Schalenknochen, lamellaren, Markknochen, Osteonknochen pro parte). Lamellar bone tissue is any bone tissue characterized by a bone matrix which is, on the whole, formed of collagenous fibers organized in a plywood-like pattern (see above).

Compact lamellar primary bone tissues of superficial (periosteal or dermal) origin are generally cellular and vascular, with, however, a vascular density often ranging from low to medium. Evidence of yearly growth cycles is commonly extensive. The period of slowest bone deposition in the yearly growth cycle is generally represented by an annulus of fine lamellar, avascular, bone tissue, commonly underlined by a resting line (L.A.G. = line of arrested growth). The zones consist of (vascular) parallel-fibred bone tissue (see above). However, if the bulk of compact bone is lamellar, superimposed bone lamellae form the yearly zone whereas the annulus is generally reduced to the L.A.G. itself. Such cyclical patterns form the basis for skeletochronology.

Following Gross (1934), it is possible to lump several categories of primary compact periosteal or dermal bone tissues under the general term lamellar - zonal bone pattern (French: tissus lamellaires zonaires, German: Zonaren Periostknochen). This broad category includes both 'psuedolamellar' (=parallel-fibred) and truly lamellar bone tissues of periosteal or dermal origin, often offering extensive evidence of cyclical growth, which can show various patterns of vascularization.

On the other hand, lamellar bone may have an entirely different origin, forming the fine lamellae of primary or secondary osteons, as well as the trabeculae of reconstructed spongy bone in the medullar cancellous region. Again, as for parallel-fibred tissue, fine lamellar bone laid down originally by the osteoblasts of the endosteum may form various structure ranging from irregular secondary trabeculae of the cancellous spongosia to secondary osteons of the inner cortex, with all possible intermediate stages. The rate of deposition of endosteal lamellar bone laid down centripetally in secondary osteons or around the medullary cavity appears to be much faster than that of subperiosteal lamellar tissues deposited centrifugally at the periphery of the cortex. Subperiosteal lamellar bone is commonly more coarsely fibred than endosteal lamellar bone.

10. Lamina bone tissue (Figures 14 and 15; French: tissu osseux laminaire, laminae 'in toto concentrico' auct.; German: laminaire Periostknochen). This is a well-differentiated tissue type within the broad category of the fibro-lamellar complex of primary compact periosteal or dermal bone tissues. In lamina bone tissue, vascularization is performed by numerous longitudinal primary osteons precisely superimposed into successive circumferential layers. Within each vascular layer, numerous anastomoses between the longitudinal osteons are formed by circularly oriented primary osteons. This dense two-dimensional meshwork of vascular canals together with the lamellar bone matrix of the primary osteons surrounding them, had been laid down in the Haversian spaces' primitively left open within the loose scaffolding of the initially deposited woven bone. Hence a complete lamina may be defined as the thickness of bone centered on one vascular layer, up to the middle of the sheet of fibrous periosteal bone surrounding it above and below.

Note that the middle of each fibrous sheet is the location of initial deposition of a new lamina by the periosteum, and it can be recognized by its denser, hypermineralized matrix, which forms a 'bright line' (Currey, 1962), the 'blasse Mittellinie' or the 'helle Mittellinie' of German authors, often associated with a few larger, flattened osteocytes ('Begleitzellen'). This 'bright line' has nothing to do with yearly lines of arrested growth (L.A.G.) as defined in skeletochronological studies. It merely reflects the 'saltatory' activity of the periosteum actively laying down new bone laminae, and is thus an accretion line (see Currey, 1960; Ricklès, 1975a) (Figure 15A).

Lamellar bone may form a large part of the massive primary cortical bone deposited during the phase of active growth in large land vertebrates. It has been described in the diaphyses of many mammals, mammal-like reptiles and dinosaurs.
Fig. 15. Typological classification of bone tissues (continued). A: The lamina of laminar bone. Diagrammatic cross section of compact laminar bone enlarged, showing three laminae. 1 (on the left), the double-headed arrow shows the definition of one lamina according to modern authors. 2 (on the right), the double-headed arrow shows one lamina according to earlier authors. Dots = fibrous bone of periosteal origin; black = lumen of vascular canals; cvPr = primary vascular canal; lc of = bright line, a hypermineralized region in fibrous bone and early site of deposition of a new lamina; of = fibrous or woven bone matrix of periosteal origin, laid down centrifugally; ol = lamellar or parallel-fibered bone matrix of the primary osteons, laid down centripetally. B: Secondary bone tissues. 1 = Scattered Haversian bone in a lamellar-zonal primary cortex. 2 = Dense Haversian bone replacing a fibro-lamellar primary cortex; note successive generations of secondary osteons and older ones as interstitial systems. 3 = Perimedullar bone remodeling; this region, the endosteal margin, makes a transition between compact cortical and cancellous medullar bone; it contains large, incomplete secondary endosteal osteons. These secondary systems are characteristic of this transitional region. 4 = Non-cortical compacta: dense deposition of secondary endosteal bone may produce thick compacted secondary modullar bone. Source: from Ricqlès (1975).

11. Plexiform bone tissue (French: tissu osseux plexiforme). This tissue type is very close to laminar bone tissue, but it is even more densely vascularized. Here, the vascular layers of each superimposed lamina are united together by radially oriented vascular canals. Hence, the vascular meshwork is three dimensional. This bone tissue pattern, of course, is linked to typical laminar tissue by an array of intermediate conditions, according to the local
number, density and regularity of the radial vascular canals. Intermediate aspects have been described as 'sub-plexiform'.

Plexiform bone tissue is well known in the cortex of the long bones of sauropod dinosaurs and in the large artiodactyl mammals. Functionally, it is linked with fast, normal deposition of large masses of new compact bone.

12. Reticular bone tissue (French: tissu osseux fibrolamellaire réticulaire). This is another variation of the general 'fibro-lamellar complex' of compact primary bone tissue. In this case, the numerous lamellar primary osteons surrounding the vascular canals have an oblique orientation and they are rather irregularly anastomosed. They are buried in a fibroblastic matrix which was initially deposited by the periosteum. This bone pattern can locally change its organization into a more orderly arrangement of the vascular canals, towards a more laminar, or plexiform, or radiating organization. This bone tissue type seems to be associated with fast deposition of relatively modest amounts of primary compact bone, perhaps in connection with special biomechanical conditions. It occurs in the compact bone of some pterosaurs and birds, but it is by no means restricted to these groups.

13. Radiating fibro-lamellar bone tissue. This is another variation of the fibro-lamellar complex, where radially oriented primary osteons take prevalence over longitudinal and (especially) circular osteons to organize the tissue. This is functionally associated with very fast deposition of relatively modest amounts of new compact bone.

14. Chondroid bone. As the term implies, this is a bone tissue which exhibits some of the histological characteristics of cartilage, but which, for reasons dealing with structure, position or function, is intermediate between 'true' cartilage and bone. The complex issue of chondroid bone has recently been extensively reviewed by Beresford (1981). This author lists several categories of chondroid bone which occur in various circumstances. Chondroid bone shows large, closely packed cells with poor canalicular development, embedded in a matrix which may show at least some of the histochemical characteristics of cartilage. Chondroid bone develops in deer antlers, which have been discussed by Hall (1975), Beresford (1981) and Goss (1983). As pointed out long ago (Weidenreich, 1930; Ørvig, 1951) and further emphasized in recent studies (Hall, 1978; Huysseune, 1986), the chondroid bone exemplifies the limits of the classical descriptive-typological approach to tissue ecology. In contrast, modern research concentrates on modifications of cells stemming from a common germinal stock. This newer approach is better suited for differentiating the full continuum of more or less closely related tissues.

15. Hyalodentine. This word was coined by Hofer (1889) for the superficial layer (or ridged layer) of elasmoid scales, which shows a "hyaline aspect". Hofer (1889) believed that hyalodentine is homologous with the dentine of ganoid scales. Modern studies have shown that the superficial layer of elasmoid scales has two layers, the external layer and the limiting layer, neither of which is likely homologous with dentine. Thus, the term 'hyalodentine' is inappropriate and must fall into disuse.

F. Osteogenic Cell Layers

1. Periosteum. The periosteum is the specialized connective tissue which covers most bones. It consists of two strata: a superficial fibrous mantle, and an active inner layer known as the cambium (Canalis and Burrstein, 1985). Kiese and Geidel (1972) described five different types of cells in the periosteum: fibroblasts, precursor cells, cambium cells, osteoblasts, and osteoblasts, in which the shape of the cell changes from longitudinal and rodlike to rounder and ovoid. All of these cells except the mature osteoblasts are capable of cell division (mitosis).

2. Endosteum. This is the layer of osteoblasts which covers the surface of the medullary cavity of a long bone and the surface of the trabeculae of endochondral bones.

3. Dermis. The dermis is the connective tissue layer beneath the epidermis. The outer surface of the dermis, in contact with the epidermis, is called the reticular layer. It consists of loose connective tissue with thin collagenous bundles (stratum laxum = stratum spongiosum). The deeper main portion of the dermis, called the reticular layer, consists of rather dense connective tissue. Its collagenous fibers form a feltwork with bundles running in various directions but, for the most part, more or less parallel to the surface (stratum compactum). See Bloom and Fawcett (1975) and Meunier and Géraudie (1980).

4. Scale-pocket (French: poche de l'écaillée). This is the dermal space which contains a single elasmoid scale. During ontogeny, the scale-pocket forms around the growing scale, near the epidermal-dermal junction. The scale-pocket is clearly delimited from the subjacent stratum compactum by the scale-pocket lining. When a scale is removed, the scale-pocket is not damaged and remains as an empty space in which a new scale regenerates. The scale-pocket associates exclusively with elasmoid scales. See Sire and Géraudie (1984) and Sire (1988).

5. Scale-pocket lining. This bilayered sheet of fibroblastic-like cells separates the floor of the scale-pocket from the stratum compactum (Whitmore et al., 1980). The cells are joined by desmosomes, and a basement membrane is located on both faces of the sheet. The cells that form this sheet have the same, still unknown, origin as the cells which form the scale (Sire, 1988).

6. Scale-sac (French: sac de l'écaillée). This is the envelope of scleroblasts (episquama, hyposquama and marginal scleroblasts) surrounding the scale and synthesizing its different parts. The scale-sac is generally uninterrupted, at least in young scales, and is located in the scale-pocket (Sire, 1988).

7. Episquama (Figure 7). This name was given by Waterman (1970) to the layer of scleroblasts located at the outer surface of the elasmoid scale of teleosts and synthesizing its external layer. The cells of the episquama build the circuli and they participate in thickening the outer limiting layer. They probably also participate in the setting of the anchoring fibers (Sire 1985b).

8. Hyposquama. This is the simple layer of elasmoblasts located at the deep surface of the basal plate (phyloplum-like structure) of the elasmoid scale of teleosts. The hyposquama synthesizes the constituents of the basal plate (Waterman, 1970). See also Sire (1975a) and Zyliberg et al. (1988).

G. Mineralization and Demineralization

1. Mineralization front (French: front de minéralisation; = ligne frontière" of Lacroix, 1971). Located between osteoid and the underlying mineralized bone, the mineralization front shows more intense staining reactions than the rest of compact bone (Lacroix, 1971; Leblond and Weinstock,
1971), especially with Soudan black B, silver nitrate (Von Kossa), and cobalt (Stoelzner). It represents the area where mineralization is initiated. At the mineralization front, glycosaminoglycans and phospholipids are more abundant, and perhaps slightly different, than in other parts of bone, in accordance with their role in the mineralization process. However, the dynamics of this mineralization process (nucleation) remains unclear (Caplan and Pechak, 1987).

2. Mandl’s corpuscles (Figure 7). Mandl’s corpuscles are calcified concretions only present in the fibriiblitate (or basal plate) of elasmobranch fish scales. Discovered more than a century ago by Mandl (1839), their first fine description is attributed to Baudelot (1873), who noted that in a given species they vary in size and shape. This variation is linked with their location, the smaller corpuscles occurring close to the scale margin and the larger ones occurring under the focus of the scale. Their size is also linked with the age of the fish. TEM and especially SEM have shown the great variability of their structure and form (e.g., ovoid, cubic, polyhedral and spherical). Their surface features reflect the arrangement of the collagen fibrils of the plywood in the basal plate of the scales (Schönborner et al., 1981; Meunier, 1984b). The morphology of Mandl’s corpuscles is more complex in the twisted plywood structure than in the orthogonal structures (Meunier, 1984b). Mineralization of the corpuscles extends farther in the direction of the long axis of the parallel collagen fibrils in each lamella (Zylinderberg and Nicolas, 1982). As the growing corpuscles become incorporated into the successively mineralized lamellae, the corpuscles show different fibril directions which correspond to the sequence of lamellae involved. Thus, the complex shape of the corpuscles is the result of the spatial organization of the collagenous plywood (Schönborner et al., 1981).

Mandl’s corpuscles play an important role in calcification of the internal layer of the scale, because this proceeds partly by fusion of corpuscles and their coalescence to the mineralizing front (Sire and Meunier, 1981).

Mandl’s corpuscles are common in teleost scales, except in the Osteoglossidae and the Mormyridae, but they are also obvious in Amia scales, where they show complex shapes (Meunier, 1981) and in Propterygii scales, which show a simpler morphology (Meunier, 1984b). In spite of their 'globular' appearance under light microscopy, calcification of the Mandl’s corpuscles is anisotropic (Schönborner et al., 1979, 1981; Zylinderberg and Nicolas, 1982). In the Osteoglossidae and the Mormyridae, the basal plate of the scales is incompletely mineralized, but the mineralization progresses quicker near the grooves than under the center of the squamaule, giving to the mineralization front a characteristic aspect (Meunier, 1983, 1984a,b). This type of mineralization is a diagnostic character which has permitted the recognition of Osteoglossidae scale fragments in fossil material (Geyet and Meunier, 1983).

3. Globuli ossei. These are more or less spherical structures inserted in hypertrophic cartilage near the resorption front of endochondral ossification. Their size is similar to chondrocytes and they show the same histological features as bone tissue. Globuli ossei apparently form around hypertrophied chondrocytes. Around each cell, the wall of the cell lacuna (chondroplast) becomes covered by a thin bone deposit that progressively fills the free space around the cell. However, the origin and nature of cells involved in this process remain unknown. Is it a migrating osteoblastic cell or a de-differentiated preexisting chondrocyte? Whatever they are, globuli ossei are very different from the spherical calcified corpuscles (calcospheres) so common in the various calcified globular cartilages of lower vertebrates (Weidenreich, 1930; Haines, 1938; Ricqlès, 1965; Meunier, 1979; Bercsford, 1981).

4. Calcification. This is a specific process of mineralization which deals with the deposition of calcium carbonate (e.g. in otoliths) or calcium phosphate (e.g. in enamel), generally in or on a pre-existing organic matrix (Moss, 1964a, 1968a; Kemp, 1984). See also Section IVG4.5.

5. Mineralization. Mineralization refers to the biologically mediated or controlled deposition of crystalline or amorphous solid mineral in or on a pre-existing organic matrix. Most biological mineralization requires specialized cells (or sclero blasts). These scleroblasts differentiate along specific patterns and they are concerned with the synthesis of the organic matrix of the mineralized structure. The organic phase has two components: a fibrous, organized one (complex polymers of proteins and carbohydrates) and an amorphous one. The unorganized phase or ‘ground substance’ is usually a viscous, highly hydrated, polyanionic colloid (Moss, 1964, 1968a).

6. Ossification. This is the formation of hydroxyapatite on a collagenous matrix in the histologically defined bony tissue; in other words, calcification of histologically recognizable osseous tissue (see also Section IIIB1 and Section IVG4.5).

7. Rate and density of mineralization. Rate (amount) of mineralization (French: taux de minéralisation) is the amount of mineral deposited per unit of bone weight. Classically this rate is obtained by measuring the weight of mineral ashes after the incineration of a known weight of dry bone. This rate is normally about 0.65, i.e., dry bone contains in weight approximately 65% mineral and 35% organic substance.

Density of mineralization can be considered in accordance with its original significance in physics, i.e. as the mass of mineral deposited per volume of tissue. However, in practice, the concept of bone density of mineralization is not so simple, notably because of variability in organic content and porosity. Consequently, it is necessary to define several concepts of density of mineralization according to the level of observation taken into account and to the methodology used. Following Laval-Jeantet (1982) one can distinguish two concepts:

a. Global or apparent mineral density (= AMD; French: densité minérale apparente). This density is the mass of mineral per unit volume of a whole bone considered as an organ (or a large piece of a bone), including the unmineralized tissues in the large cavities.

b. Tissular or true mineral density (= TMD; French: densité minérale vraie). This is the amount of mineral per unit volume of compact bone substance (mineral plus organic), excluding porosity caused by marrow sinuses, blood vessels spaces, etc.

It is clear that in compact cortical bone, the AMD and TMD have very similar values because cavities occupy only about 5 percent of the bone volume. Conversely, in cancellous bone, the AMD will be only a fraction of the TMD because the volume of bone trabeculae may be only 10 to 30 percent of the total volume of spongy bone (Laval-Jeantet, 1982).

c. Bone mineral content (= BMC; Cameron and Sorensen, 1963). This concept, often used in medical studies, is not
quite an index of mineral density itself, but is a measurement of the mass of a standardized sample of mainly cortical bone.

d. Degree of mineralization. When measurements of bone density are conducted at the fine histological level, it is better to use the concept of degree of mineralization. When precisely studied by quantitative microradiography (Sisson et al., 1960a,b; Boivin and Baud, 1984; Alcobendas and Castanet, 1985), it is possible to avoid bone lacunae and to measure meaningful fine local variations in the degree of mineralization, which is expressed as mass of mineral per volume unit of compact bone.

e. Absolute mineral density (Bergot, 1983). An even more precise parameter can be reached with the electron microprobe (e.g. Weigendal and Bylinkin, 1974) and more recently by image analysis using backscattered electrons in SEM (Reid and Boyd, 1987). In measurements of absolute mineral density, very finely localized measurements eliminate even bone microporosities (canaliculi).

8. Processes of bone demineralization. Three types of physiological processes are involved in the removal of mineral from bone tissues. The first two, osteoelastic resorption and periosteocytic osteolysis, deal with the total removal of bone tissue, both organic matrix and mineral. These two processes have been discussed in a previous section (see bone resorption C-2, above in this section).

The third process, which involves the removal of mineral without destruction of the organic matrix, was named halastatic demineralization by Ruithauser et al. (1953). 'Halastasis' is a word of Greek origin which means 'unstable salts'. More recently two modalities have been defined for this process according to its localization in bone:

a. Diffuse halastatic demineralization. This leads to a general decrease in the degree of mineralization of the whole mass of bone tissue. It has been studied by Lopez et al. (1970) for fish bone tissue, and by Baud et al. (1978) for human bone tissue.

b. Localized halastatic demineralization (halo volume; Frost, 1961, 1973). This process appears at the periphery of periosteocytic lacunae. One may note that this process occurs only in bone tissues which have already reached their optimal mineralization (Arnold et al., 1971; Frost, 1973). Thus halo volumes of demineralization should not be confused with the initial or primitive hypomineralization halos which are sometimes also observed around the periosteocytic lacunae in pathologic cases of recently mineralized bone (Buss and Frost, 1971; Baud and Boivin, 1978; Marie and Glorieux, 1983).

At the present time only a few studies claim to have shown halastatic demineralization (diffuse or localized), and authors do not even agree on the reality of this phenomenon. However, Alcobendas and Baud (1988) recently described a new type of time-dependent halo volumes which appear in winter and during the breeding period and disappear in summer in females of the snake Vipera aspis. This demonstrates that even avascular bone is involved in calcium homeostasis via bone resorption and halastatic demineralization.

V. Cells, Extracellular Matrix and Mineralization

The formation of skeletal tissues involves the synthesis of organic matrices by scleroblasts and the deposition of an inorganic salt phase in these matrices. The organic fraction represents 30 to 40 percent of the dry fat-free weight of mature bone (Eastoe, 1956). It consists mainly of extracellular matrix, because mature bone tissue is poorly cellular. Two cell types can be distinguished in skeletal tissues of mesenchymal origin. The first cell type ensures the formation, growth and maturation of the mineralized tissues. These cells arise from local mesenchyme through the osteoblastic lineage and they include osteoblasts and osteocytes. The second cell type is involved in the resorption of mineralized tissues. These are osteoclasts, which derive from the hematopoietic stem cells. Osteoclasts is a complex process related to shaping and forming mineralized tissues (Chambers, 1987).

The organic matrix is a fibrous protein with up to 90-95 percent collagen (Eastoe and Eastoe, 1954). The other minor organic components are proteoglycans, glycoproteins, sialoproteins, serum proteins and lipids (phospholipids) (Glimcher, 1976, 1981; Veis and Sambais, 1987).

The mineral phase of mature bone constitutes approximately 65 percent of the bone tissue by weight. The principal constituents of the mineral phase are calcium ions and inorganic orthophosphate ions associated with other ions such as sodium, magnesium, strontium, carbonate, citrate and fluoride. Sodium and magnesium represent a minor fraction of the mineral phase. However, this fraction represents approximately 30% and 65% of the sodium and magnesium, respectively, contained in the body. Thus, skeletal tissues represent an important physiological reservoir for maintaining homeostasis not only for calcium but also for sodium and magnesium.

A. Cells

1. Fibroblasts. These cells, which are commonly found in the connective tissue, are responsible for the synthesis of extracellular matrix components, i.e. collagen and ground substance (Junqueira et al., 1986).

2. Scleroblasts. According to the original definition by Klaatsch (1894), 'scleroblast' refers to cells lining a scale. However, subsequent workers have used this term for any type of cell involved in mineralizing a variety of biological tissues (Krejsa, 1979). Vertebrate scleroblasts are usually referred to the tissue they produce, e.g., chondroblasts for cartilage, osteoblasts for bone tissue and odontoblasts for dentine.

3. Osteoblasts. Named by Gegenbaur (1864, 1867) these are the specific cells found on the surfaces of developing bone tissue. Active osteoblasts never show mitotic activity (Owen, 1963, 1978). They synthesize bone matrix.

In ordinary histological preparations, osteoblasts are plump cells with a single eccentric, rather spherical or ovoid nucleus and highly basophilic cytoplasm. They are generally arranged as a single-layered pseudo-epithelium. Osteoblasts may vary in length from 15 to 80 microns in humans, although most fall within the range of 20-30 microns (Kelliker, 1889). Fine cytoplasmic processes extend from the cell body to make contact with its neighbours.

Electron microscopy reveals that osteoblasts possess a Golgi apparatus, the form of which closely reflects the functional state of the cell in relation to bone matrix production (Pritchard, 1952). The osteoblast cytoplasm is richly supplied with endoplasmic reticulum in the form of stacks of flattened and dilated membranous sacs (cisternae) situated on the outside with 15-nanometer granules (ribosomes) (Sheldon and Robinson, 1957). The osteoblasts also possess abundant mitochondria in the peripheral cytoplasm.
4. Osteocytes. Presumably derived from osteoblasts, osteocytes are buried within the mineralized bone matrix. They are connected to one another and to osteoblasts on the bone surface by means of canalici. Newly-formed osteocytes are very similar to osteoblasts, but when more mature, they become flatter and lose some of their abundant cytoplasm. Osteocytes vary widely in size, shape, and cytoplasmic details as well as in the density and regularity with which they are packed in the matrix (Pritchard, 1956; Cameron, 1972; Owen, 1978).

a. A periosteocytic lacuna (plural: lacunae) is the cavity enclosing an osteocyte. Some cells air at fill their lacuna, while others are separated from the surrounding walls by a zone of amorphous material and loose collagen fibrils. The average size of the periosteocytic lacuna is generally accepted as an index of osteocyte activity (Baud, 1976), although this is disputed by Marie (1985). See also Dudley and Spiro (1961) and Cameron (1972).

b. Canaliculi are fine spaces in bone tissues which house the fine, contiguous cytoplasmic processes of osteocytes and osteoblasts. The canaliculi connect the periosteocytic lacunae with one another and with the outer surface of the bone.

5. Aspidinocytes. These are fine matrix-filled spaces in fossilized aspidin, believed to have contained cells (Halstead-Tarlo, 1963). However, this assumption has promoted much discussion because aspidin, defined as a type of non-cellular bone, would not exist as a distinct type if aspidinocytes indeed exist. It seems unquestionable that some very elongated, fine, unmineralized spaces sometimes occur in aspidin, but they are probably better interpreted as cell-cytoplasmic process, or merely unmineralized matrix fibers, rather than cell spaces. See also ‘aspidin’, section IV-E-3, above.

6. Osteoclasts. Osteoclasts are a heterogeneous group of multinucleate cells (2 to 100 nuclei) with several properties in common. They are generally larger than other bone cells, ranging in diameter from 20 to 100 microns. Rich in lysosomal enzymes such as acid phosphatase and cathepsin(s), osteoclasts are found where bone is being resorbed. When in contact with the bone surface, their membranes form many processes (the brush border of Scott and Pease, 1956) which appear to penetrate the bone surface. The role of osteoclasts in bone resorption has been demonstrated both in vitro (Kahn et al., 1981; Osboby et al., 1982; Marks, 1983) and by studies of bone diseases such as osteopetrosis in which they function abnormally (Marks and Walker, 1981). Mononucleated osteoclasts are known among fishes (Hubner, 1962; Lopez, 1973; Weiss and Watabe, 1979).

7. Howship’s lacunae. These are surface depressions which characterize bone areas undergoing resorption. They represent the former position of osteoclasts (Ream and Pendergrass, 1982). Differences in the size and depth of the Howship’s lacunae have been reported to represent variations in resorptive activity (Jones and Boyle, 1970).

8. Elasmoblasts (deep scleroblasts). This name given by Meunier (1981) to the cells arranged in a simple layer, the hyposquama (Waterman, 1970), located at the deep surface of the basal plate (plywood-like structure) of the elasmoid scales. They synthesize the components of this part of the scale and they participate in the orientation of the collagenous matrix to give the plywood arrangement.

9. Elasmocytes. Elasmocytes are cells included in the plywood-like structure of elasmoid scales. However, in most of the more highly evolved teleost scales, elasmoblasts are not included in the basal plate. Elasmocytes are present in the plywood-like structure of young ganoid (palaeoniscoid) scales of the sarcopterygian lineage, elasmoblasts are found in the plywood of the scales of lungfishes and Latimeria. A cellular basal plate may be considered an ancestral condition, and the absence of elasmocytes is a derived condition. See Meunier (1983, 1984a, b) and Hsieh (1988).

10. Williamson’s canals (= lepidosteid canalici; lepidosteid tubes or tubules). These are present only in holostean bones. Described by Williamson (1849) as narrow tubes of about 3 microns in diameter, these canals penetrate into osseous tissue and are arranged at right angles to the osseous lamellae. They divide into two or more short branches at their distal extremity. Each of them contains a long process belonging to a large cell which lies between the osteoblasts on the surface of the bone tissue and retains that position by the successive elongation of its process as the tissue grows in thickness. These cells do not resemble odontoblasts in dentine and thus the lepidosteid tubules are not equivalent to dental tubules. These cells also do not resemble osteoblasts. The canals of Williamson are non vascular canals but some authors have speculated that they may be vestiges of vascular canals of various earlier fossil ray-finned fishes (Orvig, 1951). Their function is unknown but they could bring nutritive substances to the surrounding tissues. This hypothesis is supported by current ultrastructural descriptions showing that, in the canal, the cell process is particularly rich in filaments (Nickerson, 1893; Stephan, 1900; Goodrich, 1913; Aldinger, 1937; Orvig, 1951; Kerr, 1952; Moss, 1964b).

B. Extracellular Matrix

1. Collagenous components

a. Collagens. Collagens comprise a class of proteins which vary in composition and show complex structures. Nevertheless, a protein can be defined as a collagen by the presence of a triple helical domain containing peptide chains with repeating gly-X-Y triplets. The amino acid composition of collagens is characterized by a high content of glycine and hydroxyproline and the presence of a small number of hydroxylysyl residues.

The source of heterogeneity of collagen molecules is the multiplicity of collagen genes giving rise to different types of collagens which are functionally distinct. The major component of bone tissue is type I collagen. However, type I collagen, which is also the most important component of interstitial connective tissues of mesenchymal origin, shows peculiar chemical and structural characteristics unique to mineralized tissues (Glimcher, 1976; Veis and Sabsay, 1987). Bone tissues also contain small amounts of Type V collagen.

b. Type I collagen (Figure 16). Mature extracellular collagens consist of macromolecules composed of three polypeptide chains; each chain is called an alpha chain. Type I collagen contains two alpha(I) chains and one alpha(II) chain. The collagen molecule is constructed from messages transcribed from two independent genes: the pro-alpha(I) gene on chromosome 17 in humans (Huerre et al., 1982) and the pro-alpha(II) gene on chromosome 7 (Junien et al., 1982; Solomon et al., 1983). In many teleosts, type I collagen exists as an heterotrimer composed of 3 alpha-
chains: alpha(1), alpha(2), and alpha(3) (Piec, 1965). The alpha(3) chain is believed to be encoded in a third genetic locus which evolved through duplication of the alpha(1) gene (Kimura and Ohno, 1987; Kimura et al., 1987) at the time of initial adaptive radiation of teleosts. Each of the three alpha chains is coiled in a left-handed helix. The three axes of the three alpha chains rotate around one another and about a common axis in a long right-handed helix, thereby forming a coiled-coil structure. This triple helical arrangement is characteristic of the type 1 collagen molecule and gives rise to its specific wide-angle X-ray diffraction pattern (Glimcher, 1976). The length of the major triple helix of the type 1 collagen molecule is about 300 nanometers and its diameter is about 1.3 nanometers. At each end of the collagen molecule, non-helical peptides called telopeptides are sites of cross-links. In the extracellular matrix, the collagen molecules polymerize and aggregate in a highly specific fashion giving rise to collagen fibrils showing the axial repeating period (64 nanometers) observed by electron microscopy and by low angle X-ray diffraction. Characteristic light and dark 'bands' and 'interbands' can be observed in electron micrographs. The contrast between bands and the interbands may be increased by using electron dense stains such as phosphotungstic acid, lead salts and uranyl acetate.

Type I collagen alpha chains arise from precursor procollagen alpha chains with a significantly greater molecular weight than the alpha chains. The pro-alpha chains of type I collagen are cleaved enzymatically. There are minor but significant differences between soft and hard tissue type I collagens resulting from post-translational chemical modifications. These differences reflect the degree of lysyl hydroxylation, the extent and type of hydroxylysyl glycosylation, and the distribution of intra- and intermolecular covalent crosslinks.

A model for the organization of collagen macromolecules in collagen fibrils has been proposed by Hodge and Petroska (1963). This model, modified by Katz and Li (1973), Glimcher (1976), and Lees et al. (1984b), currently favors a 'quasi-hexagonal packing scheme'.

Collagen fibrils are the basic structural components in extracellular bone tissue. They may be aggregated into large units in highly specific arrangements to form collagen fibers, and the collagen fibers can be packed into larger units or fiber bundles. Collagen fibers and fiber bundles are distinguishable by light microscopy. Higher orders of organization reflect the spatial arrangement of the fiber bundles and give rise to the basic types of bone tissues (see below, in section IV).

c. Type V collagen. Type V collagen is widely distributed among different tissues and has been characterized chemically in the past few years (Broek et al., 1981). Like other interstitial collagens, type V collagen consists of three alpha chains forming a triple helix. Most Type V collagen
macromolecules seem to be composed of two alpha1(V)
chains and one alpha2(V) chain. It has been shown that
procollagen type V consists of three distinct pro alpha chains:
pro-alpha1(V), pro-alpha1(V), and pro-alpha2(V) (Fessler et
al., 1981). However, a third alpha3(V) chain has been
identified in several tissues (Fessler et al., 1983).

The exact arrangement of the type V collagen fibrils in
the matrix has not been established. It appears as thin fibrils associated with thicker type I collagen fibrils (Birk et
al., 1988). Type V collagen may play a role in the
mineralizing matrix, but it appears to have little effect on the
issues once these become mineralized (Veis and Sembay,
1987).

d. Elastoidin. This macromolecule characterizes the
ceratotrichia of Selachians and the actinotrichia of the
Osteichthyes. Observed with the transmission electron
microscope, elastoidin presents the typical striation of
collagen fibrils. Dense bands are regularly found every 60-
65 nanometers. In addition, 5 sub-bands exist between each
pair of dense bands. This structural organization shows that
elastoidin is not a typical striated collagen: indeed, chemical
analyses show that a non-collagenous protein is associated
with the collagen moiety (see review by Mathews, 1975).
Elastoidin comprises three identical alpha chain subunits
(Kimura et al., 1986); it is closely related but nevertheless
distinct from skin type I collagen. The non-collagenous
protein, rich in tyrosine and cysteine, is also glycosylated
(i.e., it contains galactose and glucosylgalactose).

There are presently no data on the biochemical
composition of the elastoidin found in the actinotrichia of
Osteichthyes. At present, it is inferred to be homologous on
the basis of the similarity of its fine structure.

2. Non-collagenous components (Figure 17)

Various non-collagenous components identified in bone are
represented by a wide spectrum of molecules, the precise
roles of which in mineral deposition remain incompletely
known.

a. Proteoglycans. Proteoglycans (PG) are macromolecules
containing 10 to 50 or more characteristic carbohydrate
polymers, glycosaminoglycans (GAGS). Each polymer is
bound covalently to a core protein chain. The PG are
interrelated to the collagen fibrils. Electron microscopic and cytochemical studies have shown that the
attachment points of proteoglycans on the collagen fibrils are
periodic, they associate with collagen fibrils in the hole
regions of the collagen type I fibrils (Laras and Cooper,
1972).

The mineralization of bone matrix is accompanied by a
dramatic loss of PG (Baylink et al., 1972). Degradation of
PG appears to be the prelude to mineralization of the
collagenous extracellular matrix by rendering the collagen
matrix more accessible to the mineral ions. Thus, the PG
probably regulate mineralization by acting as inhibitors of
the deposition of solid phase Ca-P in collagenous tissues.
However, the exact mechanisms of their action are still open
to question (see Gimcher, 1976; Blumenthal, 1981; Fisher
et al., 1983; Caplan and Pechak, 1987; and Posner, 1987).

b. Sialoproteins. These proteins consist of a sialic acid
(N-acetyl neuraminic acid) attached to a non-collagenous
protein to short oligosaccharide chains. Sialoproteins are
found in developing bone and compact bone (Sato et al.,
1985). The function of these proteins is presently unclear
(Gehron-Robey et al., 1988).

c. Glycoproteins. Glycoproteins in bone extracellular
matrix have shorter carbohydrate chains than proteoglycans
(Hemm, 1972).

d. Phosphoproteins. In these non-collagenous proteins,
phosphate is covalently bound to the protein backbone at the
level of serine and/or threonine amino acid residues.
Osteocalcin is the best chemically characterized phosphory-
lated bone glycoprotein (Gehron-Robey et al., 1988).

e. Osteocalcin (bone gla-protein). Glycoprotein is
characterized by its gamma carboxy-glutamic acid (gla)
residues. These g1a residues show an affinity for calcium
ions. Osteocalcin appears in developing bone coincident
with the onset of mineralization (Bianco et al., 1985).
Osteocalcin is supposed to be involved in bone turnover and
remodeling (Reddi et al., 1981). The presence of osteocalcin
has been demonstrated in fossil bones and teeth (Ulrich et
al., 1987).

f. Osteonectin. This glycoprotein was isolated from
mineralized tissues by Termine et al. (1981). Osteonectin
binds collagen and hydroxyapatite. It has been proposed that
osteonectin directs the specific site of nucleation and growth
of apatite crystals in newly formed mineralized tissues (Sato
et al., 1985, Lund et al., 1987).

g. Serum proteins. In mineralized bone, serum proteins
such as serum albumin, alpha2-HS-glycoproteins, transferrin
and immunoglobulins have been identified. Their functions
are unknown (Gehron-Robey et al., 1988).

h. Lipids. Lipids have been detected in bone (Wuher,
1968), particularly phospholipids. Calcium-acidic phos-
pholipid-phosphate complexes have been isolated from bone.
They are more highly concentrated in the mineralizing front
(Boskey, 1978; Posner, 1987). The acidic phospholipids are
supposed to play a role in the initial deposition of
hydroxyapatite (Boskey, 1981).

C. Mineralization (Figures 19, 20)

1. Hydroxyapatite. Hydroxyapatite is the characteristic
calcium phosphate mineral in vertebrate skeletal tissues
(Selvig, 1970). With the exception of enamel, the mineral

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**Fig. 17.** Approximate percentage of non-collagenous bone
matrix proteins extracted from bovine subperiosteal bone.
Source: from Gehron-Robey et al. (1988).
Regardless of the examination procedures, the crystals appear irregular in shape (Jackson et al., 1978). In human adult bone, the largest crystal dimension varies from 25 to 35 nanometers and their thickness ranges from 2.5 to 3 nanometers (Posner, 1987). The sizes and the shapes of the crystals do not change significantly during mineralization of the tissues (Landis et al., 1977b).

The hydroxyapatite is preceded by an initial precursor, the identification of which remains one of the unsolved problems in skeletal biomineralization (Blumenhal and Posner, 1973; Glismer, 1981, 1984; Glismer et al., 1981; Weiner, 1986; Posner, 1987). It has been suggested that the precursor consists of a non-apatitic calcium phosphate which might be amorphous calcium phosphate (ACP) (Termin and Posner, 1967; Banes et al., 1967, 1973). The precursor would be transformed into poorly crystalline hydroxyapatite via intermediates such as octacalcium phosphate (OCP: Ca₅(H₂PO₄)₂(PO₄)₂·5H₂O) (Brown, 1966), and brushite (CaHPO₄·2H₂O) (Roufosse et al., 1979) or whitlockite (McConnell, 1973; Driessen and Verbeek, 1986).

2. Minor components of the mineral phase. In addition to calcium and phosphate, the mineral in skeletal tissues contains carbonate, citrate, fluoride, and traces of several other ions (Mg, Sr, Mn, Ba, Na, etc.) which are present in hetero-ionic exchange with or without the apatite structure (Engström, 1972; Montel, 1977; Posner, 1987).

a. Carbonate: After calcium and phosphate, carbonate is the third most important chemical group found in bone apatite. Carbonate fluorapatite (francolite) contains 4.5 percent carbonate by weight, and bone contains about as much carbonate as does francolite (McLean and Urist, 1961). The position of carbonate in the mineral is still debatable. It is believed that about half of the carbonate in bone is merely held on surfaces and is easily exchangeable; the other half would be located in the phosphate position and would be responsible for an increased crystal reactivity (Weiner, 1986; Posner, 1987).

b. Fluoride: Fluoride ions substitute for OH ions in fluorapatite. The OH and the F positions differ slightly in the crystal lattice (Posner, 1987). A larger crystal size is observed in fluorapatite compared with hydroxyapatite; concomitantly, these larger crystals show decreased solubility in water and they appear to be more resistant to resorptive influences (Boivin and Baud, 1978; Posner, 1987).

c. Magnesium: Magnesium is one of the most important minor inorganic constituents in bone. It has been hypothesized that magnesium is preferentially excluded from the apatite lattice (Neuman and Mulyan, 1971) and associates instead with the crystal surface. However, the precise location of magnesium ions within the mineral phase is still an open question (Terpstra and Driessen, 1986). The accumulation of magnesium in mineralizing tissues suggests that it affects the nucleation and growth of hydroxyapatite crystals (Posner et al., 1977) through involvement in the control of acidic phospholipids (Bosskey and Posner, 1980).

d. Silicon: Silicon has been considered a critical factor for normal skeletal development (Carlisle, 1970). Silicon appears to be located in the well-mineralized extracellular matrix where poorly crystalline hydroxyapatite is found associated with inorganic fluorapatite (Landis et al., 1986).

3. Matrix vesicles (Figure 18). These are small, round (25-200 nanometers in diameter), extracellular, membrane-limited bodies (Bonucci, 1967; Anderson, 1969). Matrix vesicles containing glycoproteins and lipids have been identified in the earliest sites of mineral formation in several types of calcification (Bonucci, 1970; Hale and Wuthier,
The problem of the function of the matrix vesicles is not yet resolved. In the context of a "chemical machinery" allowing the nucleation of hydroxyapatite (Bonucci, 1984), the matrix vesicles may be the sites of initial nucleation of the mineral deposit. However, calcification is known to occur in the absence of matrix vesicles, thus, matrix vesicles cannot be considered the exclusive mechanism for crystal nucleation in skeletal tissues (Vavasseur, 1982; Ali, 1983; Glimcher, 1984; Bonucci, 1987; Selan et al., 1987). Because matrix vesicles are more numerous in the early stages of bone development during bone repair, their presence might be related to rapid mineralization (Glimcher, 1984; Selan et al., 1987). According to Bonucci (1979), sites of clusters of crystals called "crystal ghosts" represent the initial sites of mineral nucleation.

4. Fine mineral localization (Figures 19, 20). In vertebrate skeletal tissues, the orientation of the mineral deposit is related to the nature and the morphology of the organic matrix (Landis, 1981). Electron micrographs of bone show the hydroxyapatite crystals oriented along the collagen fibrils such that their long axes are aligned with the axes of the collagen fibrils (Höhling et al., 1971; Kesting et al., 1980). The alignment of the crystals along the collagen fibrils is in accordance with the model established by Hodge and Petruska (1963). A close genetic association of mineral and collagen is supported by the fact that collagen can nucleate hydroxyapatite (Glimcher, 1976). This process is regulated by phosphoprotein (Glimcher, 1984; Posner, 1984). The mineral deposit accumulates the characteristic periodic striation of collagen fibrils because the initial sites of hydroxyapatite correspond to the "hole zones" between the tropocollagen molecules packed within the collagen fibrils. According to Hodge and Petruska (1963) and to Glimcher and Krane (1968), the hole zones provide enough space for about 50 percent of the mineral phase of bone. More recently, Lees et al. (1984a) estimated that the intrafibrillar mineral reaches at most 20 percent of the total mineral. In bone, it has been shown that the mineralization of the collagen fibrils precedes that of the interfibrillar spaces. However, in the osteoid, the crystals are associated in clusters which appear randomly distributed in the extracellular matrix and their relationship with the collagenous network is not evident. In other skeletal tissues such as fish scales, crystals of hydroxyapatite are deposited first in the extracellular matrix around the collagen fibrils with which they are contoured. The mineral does not appear to invade the collagen fibrils even in the late stages of mineralization (Onoizato and Watabe, 1979), even under examinations by TEM using anhydrous techniques (Zylosberg and Nicolas, 1982). In the early stages of experimental cutaneous calcinosis, the crystals of hydroxyapatite are also located mostly in the extracellular matrix, around the collagen fibrils (Bavilin, 1975). Thus, the question of the precise location of the mineral within, on or among the collagen fibrils remains unanswered.

a. Isotopic mineralization is a type of mineralization characterized by specific interactions between collagen fibrils and the deposition of the mineral phase (Orvig, 1968, and see above). Isotopic mineralization is the usual process in vertebrates (Moss, 1961) in comparison with spheric mineralization, which is rare.

b. Spheric mineralization is characterized by the formation of mineralized globules within which the crystals and the organic matrix show a radiating arrangement. In vertebrate skeletal tissues, spheric mineralization may be as a phylogenetic precursor of isotropic mineralization, the latter possibly representing the "ultimate stage" in the phylogeny of calcification mechanisms (Orvig, 1951, 1968). Spheric mineralization has been observed in the postcranial dermal skeleton of lower vertebrates where it coexists with the isotropic type (Zylosberg et al., 1980; Levrai-Calviac and Zylosberg, 1986; Sire, 1987). It is also well
developed in cartilages (Ørvig, 1951) and in some dentines (Schmidt and Keil, 1971).

VI. Non-osseous Hard Tissues

Vertebrate non-osseous hard tissues are highly varied, but many of the observations offered about bone (see above) apply to them as well. With the exception of cartilage (or rather the family of cartilaginous tissues), all of the non-osseous hard tissues described above are restricted to vertebrates. Ligaments and especially tendons ossify by direct mineralization, and they are sometimes involved in erosion/deposition cycles, giving rise to dense Haversian bone tissues.

Most other non-osseous hard tissues occur in the superficial dermal skeleton, forming scales in the lower vertebrates and tooth components in both the lower vertebrates and tetrapods. The most important of these tissues comprise the dentine family. Cementum, which is a periodontal tissue, is very close to bone histologically.

Entirely different from the preceding tissues, all of which have collagenous fibers in their matrices, are the hard tissues of the enamel family, which have non-collagenous matrices synthesized by epidermal cells. However, all these tissues share the same basic mineral component, small crystals of hydroxyapatite, which show various relationships with the fine spatial organization of the matrices.

Finally, non-skeletal hard tissues, such as calcium carbonate inner ear concretions in fishes, have proved of great interest for studies of growth and aging.

A. Skeletal Non-osseous Hard Tissues

1. Cartilage (Figures 8, 9, 21). Cartilage is a specialized type of connective tissue in which there is usually no contact between cells. Cartilage contains a homogeneous cell population which secretes specific structural macromolecules, such as type II collagen fibers and special proteoglycans, and it is characterized by low cellularity and
low use of oxygen. Cartilage metabolism is basically anaerobic (Stockwell, 1979). Cartilage is classically characterized by a lack of blood vessels (except the cartilage canals), nerves and lymphatics. Functionally, an outstanding characteristic of cartilage is its ability to grow both from within (as a result of cell proliferation and production of new matrix) and by subperichondral apposition of new cartilage at the periphery of the cartilaginous mass. Well-adapted to high growth rates during early ontogenesis, cartilage has been regarded as a 'caenogenetic tissue', a specialized tissue for early (embryonic) stages of development.

Cartilage forms the earliest model of endoskeletal elements, both before and during ossification. These models attain shapes which are grossly predictive of the future bones which replace them in the adult. Epiphyseal, metaphyseal and diaphyseal regions can be recognized, and the entire cartilage becomes surrounded by a perichondrium, or chondrogenic membrane. After growth and ossification, permanent cartilages may be restricted to the thin articular cartilages capping the epiphyseal surfaces.

Anatomical terms and concepts referring to cartilaginous young bones (i.e. cartilaginous epiphyses, growth plates) which are morphological entities should not be confused with histological terms (i.e. hyaline cartilage, hypertrophied cartilage), which deal with an entirely different level of integration.

Many groups of vertebrates retain more or less important parts of their endoskeleton as cartilage throughout their life, especially water-dwelling forms.

a. Perichondrium. This is a special layer of connective tissue which covers cartilage (Bloom and Fawcett, 1975). Its superficial region (fibrous layer) is rich in type I collagen fibers and elastic fibers, whereas its deeper part (chondrogenic layer) merges with the cartilage itself. There, chondroblasts frequently divide and produce matrix, thus becoming chondrocytes. Hence, the periosteum is responsible for cartilage accretional growth. The perichondrium is well vascularized. Perichondrium is a transitional layer during ontogeny because it is replaced by the perios- teum on growing diaphyses up to the 'encoche d'ossification' (see Section III-A-1-c, above). However, its fibrous layer is importantly involved in metaphyseal regions with the early differentiation of tendinous and ligamentous insertions.

b. Chondroblast. This represents the first stage of mesenchymal cell differentiation in the process of cartilage production, during both ontogenesis and fracture repair in the adult. Chondroblasts usually do not produce the type II collagen molecule which is considered characteristic of cartilage, but rather type I collagen (Kosher et al., 1986). They differentiate in early precartilaginous condensations and, later on, in the inner region of the perichondrium membrane, ultimately giving rise, by mitoses, to chondrocytes.

c. Chondrocyte. This is a fully mature cartilage cell located in a chondroplast and immersed within the extracellular gel-like matrix which it produces. This matrix contains type II collagen fibers and a specific sulfated proteoglycan. A differentiated chondrocyte is round or polygonal, and its cytoplasm contains numerous cisternae of rough endoplasmic reticulum. Golgi centers and secretory vesicles. Chondrocytes maintain their potential for active division by mitosis, each daughter-cell producing new matrix. Hence cartilage is rather unique among skeletal tissues in its ability to grow by expansion from within.
(intussusceptive growth), as long as the matrix has not become calcified.

d. Chondroplast (‘chondrocyte lacuna’). A chondroplast is the space between the ruffled plasma membrane of a chondrocyte and the extracellular matrix which it secretes. It contains enzymes and structural molecules involved in the construction of the matrix.

e. Chondroblast. This is a specialized cell, generally (but not necessarily) a giant multinucleated one which destroys by phagocytosis most of the hypertrophied cartilage (cells and matrix) at the level of the erosion front which delineates the marrow cavity in young bones. Chondroblasts are cytologically similar to osteoblasts and they have analogous functions, although their specialized activity restricts their location near cartilage.

f. Articular cartilage. This is a permanent mass of cartilage present at the joint regions between bones. It is especially well developed at the epiphyseal surfaces of the long bones of the limbs. Of the two cartilages which form a joint (articulation), one is generally concave and the other convex. They work against each other under pressure within an articular pocket (derived from perichondrium) lubricated by synovial fluid. Histologically and cytologically, articular cartilage consists of chondrocytes and non-mineralized matrix.

Between the superficial cells, collagen fibers (type II collagen) are organized parallel to the articular surface. Below these cells, the fibers may run directly perpendicular to the surface toward deeper regions of the epiphysis (if located on a long bone). In the superficial region, chondrocytes are flattened cells, parallel to the free articular surface. Cell proliferation here is a rare event but it can be induced experimentally (Kember, 1983). In the deeper region, opposed to the articular surface, chondrocytes acquire a direction orthogonal to the surface. There the cartilage may calcify and the cells, which resemble bone cells in their development of fine cell processes, are trapped in vertical rows in the calcified matrix. This coat, described as metaplastic bone by Haines and Mohuiddin (1968), most commonly forms the ‘epiphysial surfaces’ in fossils (Ricqlès, 1975a). Deeper, it is replaced by the endochondral bone of the epiphysis.

g. Growth cartilage. Growth cartilages include all cartilage masses in young (or adult lower vertebrate) skeletons not directly involved in joints (articulare cartilages). In growing long bones, they form the main masses of the epiphyses and metaphyses, down to the erosion front where endochondral ossification takes place. In several groups of vertebrates (bony fishes, urodeles, chelonians, archosauromorph reptiles, etc.) growth cartilages form an uninterrupted mass from the articular cartilage down to the marrow cavity in the diaphysis. In other vertebrates, it is interrupted by a more or less well developed secondary center of ossification which develops within the epiphysis. In these instances the growth cartilage is divided into a subarticular region in the epiphysis and a metaphysial growth plate (the ‘cartilage de conjugaison’ of other authors) between the intraepiphysial secondary center of ossification and the erosion front in the metaphysis (in anurans, most lepidosaurian reptiles, and mammals).

Within the growth cartilage one can observe various histological regions resulting from the progressive differentiation of chondrocytes in space and time. Hence, hyaline, seriated (or stratified) and hypertrophied (and calcified) cartilages, arranged in this order from the epiphyseal surface towards the diaphysis, are merely regions where chondrocytes have reached a common stage of differentiation and specific physiological activity. Overall, those successive stages of chondrocyte differentiation collectively account for growth in length of long bones.

h. Cartilage canals. These are channels of vascularized mesenchyme which penetrate the hyaline cartilage of long bone epiphyses prior to epiphyseal ossification (Stockwell, 1979; Kuettner and Pauli, 1983). Cartilage canals contain an artery and a vein which play roles in cartilage metabolism, plus unmyelinated nerves. In adults, cartilage canals persist in the permanent costal, tracheal or laryngeal cartilages.

i. Hyaline cartilage. Hyaline cartilage forms part of the cartilaginous epiphyses of young growing ‘higher’ vertebrates, and the permanent cartilaginous epiphyses of ‘lower’ vertebrates. It is histologically a mature cartilaginous tissue at a stage just before the cells begin their ultimate maturation towards hypertrophy.

Hyaline cartilage is recognized by its abundant, homogeneous matrix and its metachromatic staining with toluidine blue or alcian blue at low pH. This reflects the presence of abundant polyanions (sulfated glycosaminoglycans) in the matrix, which remain non-mineralized.

The constituent chondroblasts or chondrocytes are more or less spherical and scattered in the matrix. They actively divide. Cytologically, at the EM level, the extracellular matrix contains granules (presumably the proteoglycans) and numerous fibrils (with or without striations with a 60- to 62-nanometer periodicity) representing basically type I collagen.

j. Seri rate cartilage (= serial cartilage, zone of flattened cells, stratified cartilage zone). In seri rate cartilage, the chondrocytes are disposed in long, parallel columns separated by narrow bands of matrix. Chondrocytes form isogenic groups which form columns resulting from successive divisions of a single chondrocyte. This accounts for the interstitial growth in length of the cartilage (Bloom and Fawcett, 1975). Seri rate cartilage occurs in the epiphyseal plate of long bones and between the region of hyaline cartilage and hypertrophic cartilage. The rate of longitudinal growth is linked to the amount and regularity of the axial isogenic groups. This rate is species-specific and varies within species with age. Serial cartilages organized in well-marked axial isogenic groups are very well developed in fast-growing metaplasms of young mammals and birds. They are far less differentiated in homologous regions in urodeles and chelonians, where they have been called stratified cartilage.

Finally, below the articular surface in tetrapods devoid of secondary epiphyseal centers, and towards these centers in metaphysial growth plates of mammals, one can observe a special pattern of differentiation of serial cartilages into coronal isogenic groups, the daughter-cells being organized into spherical patterns in the matrix. This process contributes primarily to lateral growth of epiphyseal regions, and only poorly to overall growth in length.

k. Hypertrophic chondrocyte and hypertrophic cartilage zone. A hypertrophic chondrocyte is a large cell located in the zone of hypertrophic cartilage which differentiates in the region of active longitudinal growth of a long bone. In addition to its large size, it is characterized by its high glycogen content, a storage form of sugar. The latter may be involved in cartilage calcification in association with the enzyme phosphorylase, producing the phosphorylated
sugars in calcifying cartilage. Crystals of hydroxyapatite (calcium phosphate) are deposited along the (longitudinal) walls of the hypertrophied chondroblasts in the extracellular matrix containing type II collagen and proteoglycans.

Most hypertrophied cartilage (both cells and calcified matrix) is destroyed by chondroclasts at the 'erosion front'. The remaining trabeculae of calcified cartilage matrix which survive erosion form the 'director trabeculae' which are the endochondral bone is finally deposited. This process of substitution of cartilage by bone (neoplasia) is typical of endochondral ossification in mammals (Pritchard, 1972) and is classically accepted as the general rule for any endochondral ossification. However, this generalization is perhaps ill-founded, as suggested by many observations of lower vertebrates (Bloom and Fawcett, 1975).

1. Kastenschenko's line. This term was used by Haines (1942) for a peculiar structure in long bones, first described by Kastenschenko (1881) for growing animals. When chondroblasts destroy the hypertrophic cartilage in the young diaphysis to make room for the marrow cavity, they may not destroy all the cartilage matrix located at the periphery of the diaphysis, just under the periosteal bone. Thus, when endosteal bone is later deposited centripetally around the marrow cavity, it remains distinct from the periosteal bone laid down centrifugally, separated by this thin cost of cartilage matrix, which forms Kastenschenko's line.

Kastenschenko's line (not to be confused with typical growth lines in periosteal bone) can be useful for skeletal chronological studies because it provides clear evidence that the first periosteal bone deposit (in young growing individuals) has not been resorbed by perimembrane osteoclasts. Thus the complete sequence of periosteal bone deposition is preserved during later ontogeny, and 'growth rings' should therefore provide a direct expression of age in years. Kastenschenko's line is best known in the long bones of amphibians. See also Ricqlès (1965) and Francillon (1981).

2. Calcified cartilage (= mineralized cartilage). This occurs in epiphyseal and metaphyseal regions of long bones, but it is also found in the short bones of the skeleton. It represents the initial centers of calcification and provides sites for future bone formation via endochondral ossification.

Calcified cartilage results from the deposition of a solid mineral phase in an organic matrix. A variety of physiological processes may be involved. Apatite crystals may be formed de novo if the ionic environment is adequate (Ali, 1983). However, the role of chondrocytes and collagen fibers in the mechanism of calcification remains unclear. The presence of proteoglycans as inhibitors of calcification is also controversial. On the other hand, phospholipids and enzymes such as alkaline phosphatase and carbonic anhydrase are probably involved in the calcification of cartilage because their distribution parallels that of calcification. It is noteworthy that, unlike the inorganic calcification of bone, calcification in most cartilages is spherite. In spherite mineralization of cartilage, fine hydroxyapatite crystals are arranged around a calcification center, thus forming expanding globules (calciospheres) which may fuse to form larger masses. The spatial organization of the crystals is independent of the locally present matrix, notably collagenous fibers (örvig, 1951; Schmidt and Keil, 1971). Chondrochthyan, however, may have special mineralized cartilages (prismatic, areolar; see Orvig, 1951) where mineralization may not be spherite but rather inorganic and more akin to bone (Kemp and Westrin, 1979). The amount of mineralization in cartilage (mass of mineral per unit volume of tissue) is generally higher than in bone.

3. Fibrocartilage. This occurs in the intervertebral disk and in sites of attachment of tendons to bones. This type of cartilage may cover hyaline secondary cartilage, such as in the mandibular condyle. Its matrix contains large collagen fibers organized into large bundles connected by smaller ones. They may represent type I or a mixture of type I and type II collagen fibrils. Fibrocartilage is often considered to be a "transitional form between cartilage and dense connective tissue" (Bloom and Fawcett, 1975).

4. Secondary cartilage. Especially found in the mardular condyle of mammals, this was described as "fibrocartilage or dense fibrous connective tissue" by Boyd and Jones (1983). Round chondrocytes are immersed in large bundles of collagen fibers. It is believed to be a slowly growing cartilage which may mineralize by way of calciospheres. Secondary cartilages develop in the dermal skeleton of embryonic birds and mammals, notably in regions functioning as hinges.

5. Elastic cartilage. This occurs in the adult human ear (auditory and eustachian tube epiglottis) and it forms 'accessory' organs around joints (knees), commonly in association with fibrocartilage. In elastic cartilage, the chondrocytes are isolated round cells which eventually become organized into isogenic groups. The extracellular matrix contains a dense network of large fibers of elastin, a molecule differing from collagen in containing only type II collagen fibrils (Mayne and Von der Mark, 1983). This type of cartilage never mineralizes and appears to form regions submitted to opposite and alternating mechanical strains (compression versus traction and shearing).

6. Endoskeletal tesserae (tessera is Latin for 'square' or an element in a mosaic). Endoskeletal tesserae are discontinuous calcified prisms separated by uncalled cartilage. They vary in size and shape and are commonly irregularly polygonal. At higher magnifications, the outer surfaces of tesserae are very rough and can show globular excrescences or calciospheres. Endoskeletal tesserae show outer and inner zones that develop specific histological features (Tremjakoff, 1926; Kemp and Westrin, 1979). The outer zone is penetrated by collagen fiber bundles which are anchoring fibers and contain more or less aligned spindle-shaped cells. These cells may derive directly from the perichondrium. In the inner zone, round and randomly distributed chondrocytes are trapped by calcification of the cartilage. These tesserae grow in all directions, adding mass in their outer, inner and lateral surfaces (Kemp and Westrin, 1979). Although the tesserae contact each other laterally, they remain partially separated by uncalled cartilage and hence retain their potential for lateral expansion. Yet, some tesserae, according to the species, can fuse together. This tesserae pattern provides three obvious advantages for the skeletal system: support, flexibility and the opportunity for growth without continuous remodeling, unlike bone (Kemp and Westrin, 1979).

2. Ligaments. Ligaments are fibrous structures which connect two adjacent skeletal elements. They may or may not allow mobility. Ligaments consist of parallel bundles of collagen fibers in which a few elastic fibers are present (Gardner et al., 1960; Bloom and Fawcett, 1975).

3. Tendons. A tendon is a band of connective tissue which attaches a muscle to an element of the skeleton in order to promote movement (Gardner et al., 1960). As in ligaments, tendons consist of collagen fibers (Trelstad and Hayashi,
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1979; Squier and Bausch, 1984) which merge with the perimysium of the muscle at one end, and the periosteum of the bone at the other end (Bloom and Fawcett, 1975).

Tendons are sometimes capable of mineralization, especially in the necks and legs of birds (Hall, 1978). For example, the tibio-tarsus tendon is frequently used as a model to study apatite mineralization of collagen (Krefting et al., 1980; Landis, 1986).

4. Ganoine. Ganoine was defined by Williamson (1849) as the dense, homogenous enamel-like substance covering the upper (exterior) part of ganoine scales. The origin of ganoine (epidermal, dermal or mixed) has been the subject of much controversy. A recent study of regenerating polyodontic scales has shown that ganoine is directly produced by inner epidermal layer cells (= ameloblasts) after dentine has formed at the scale surface (Sire et al., 1987). The organic matrix, called preganoine, consists of thin fibrillar and globular materials deposited perpendicular to the scale surface. During maturation, the preganoine mineralizes progressively to produce the hypermineralized layer of ganoine. A new layer of ganoine is deposited with each new growth stage of the scale. Ganoine shows on its surface regular tubercles which may have a mechanical function (Meunier et al., 1982). See also ‘enamel’ (sections VI.A, VI.A) and ‘ganoine scales’ (section I.B.3.d).

5. Enamel (French: émail; German: Schmelz). Enamel is a highly mineralized layer deposited by ectodermal ameloblasts after dentine formation has started. Its mineral crystals are more or less perpendicular to the depositional surface. Enamel occurs in the surface layer of dental bones and scales of Crossopterygii and Dipnoi, and it forms the surface of the enamelite in the scales of extinct Sarcopterygii. The ganoine covering the dental skeleton of polypterids and lepisosteids is a kind of stratified enamel (Sire et al., 1987).

6. Enameloid (= mesodermal enamel; modified dentine; vitrodentine; durodentine). Enameloid is an ectodermal and mesenchymal substance deposited prior to dentine formation. The organic matrix is produced by the most superficial part of the dental papilla and is ectomesenchymal in origin. The problem of the origin of the enameloid (enamel-like) substance covering the dental plates of Osteostraci and Heterostraci and the placoid scales of Chondrichthyes has long been debated. Much has also been published regarding the enameloid of fish teeth. Experiments using amino acids have shown that the inner dental epithelium secretes a protein which interacts with the enameloid matrix formed by the odontoblasts. Tetrapod enamel appears to be chemically closely related to enameloid in elasmobranchs and teleosts. See also Halstead-Tarlo (1965), Ørvig (1967), Poole (1967), Moss (1970), Shells and Miles (1974), Schaeffer (1977) and Herold et al. (1980).

7. Dentineous tissues and dentine. Various groups of extinct and living fishes show a greater diversity of dentine architecture than do tetrapods. Dentine can be cellular (= dentineous tissues) or acellular (true dentine):

a. Dentineous tissues, or cellular dentines, may form as a consequence of rapid odontogenesis. The main types are mesodentine and semidentine. Mesodentine is characterized by cell space components having two or more branching and commonly interconnecting tubules of irregular shape. The cells are intermediate between osteoblasts and odontoblasts. This occurs in the dental skeleton (odontodes) of Osteostraci and Acanthodii. Semidentine has single dentinal tubules, sometimes with small distal and proximal branches, joined to rear-drop cell spaces situated at distances from the vascular canals. The cells are odontoblasts. Semidentine occurs primarily in arthrodirans (Ørvig, 1967).

b. True dentine (= mesodentine sensu Ørvig 1967), which is acellular and occurs principally in teeth, has numerous variants. Orthodentine is a compact dentine surrounding a pulp cavity. Orthodentine consists of an outer layer (pallial dentine) with a more or less coarsely bundled matrix and an inner layer (circumpulpar) with a parallel fibered matrix. It commonly occurs in the odontodes of Heterostraci. Osteodentine (= trabecular dentine) may consist of concentrically lamellated dentineous structures similar to primary osteon, but consisting of dentine instead of bone) with a parallel-fibered matrix surrounding vascular canals, or it may occur as interstitial zones of woven-fibered bone tissue between the dentoons. See Ørvig (1951, 1967, 1969, 1975), Schultze (1969) and Denison (1974).

8. Pleromic hard tissues (= plerom). This is a hypermineralized dentinoid tissue which has evolved convergently in several groups. It is similar to enameloïds but clearly differs from them structurally. Pleromic hard tissue develops in cavities of dental plates on preexisting dentine or on bone trabeculae. The depositing cells resemble odontoblasts. This may be interpreted as a product of the fusion of odontodes in a vertical direction. It also occurs between odontodes in the dental bones of certain Heterostraci (Ørvig, 1967, 1976a, 1980; Reif, 1973; Smith, 1984, 1985).

B. Non-skeletal Non-osseous Hard Tissues: (Otoliths)

Otoliths, or ‘ear-stones’, are acellular mineralized concretions which occur in the inner ear in all Actinopterygii except the Chondrostei. In all probability they are commonly the first calcified structure to appear in the early ontogeny of teleosts. Otoliths occur in three pairs, but in the specialized literature the term otolith is commonly but incorrectly applied to the largest of the three otoliths in the fish labyrinth; this otolith called the sagitta (=saccolith), is contained in the saccus. The two other otoliths are the lapillus (in the utriculus) and the asteriscus (in the lagenæ), the former being the largest of the three otoliths in numerous Osteichthyes. Otoliths are organs of equilibrium in theotic apparatus, rather than parts of the skeleton. In the following discussion ‘otolith’ is used as a synonym for ‘sagitta’.

Generally, otoliths are relatively flattened and they show two faces. The outer face, flat or concave, is amorphous and may show concentric rings, whereas the inner face, flat or convex, shows various sculpturing. Morphological details of otoliths are commonly diagnostic at the species level. This fact is useful for various scientific studies including nutrition of ichthyophagous predators, comparative ichthyology, paleoichthyology, and archeology (Casteel, 1976; Nolf, 1986).

Otoliths show continuous growth during the life of the fish. Otoliths grow by incremental centrifugal accretion through differential deposition of calcium carbonate (aragonite) and protein (otolin). Under high magnifications the long axes of the crystals are observed to be oriented orthogonal to the growth increments, and they are enmeshed in a proteinaceous matrix, a band of which apparently terminates crystal growth at each end (Dunkelberger et al., 1980).
Reibisch (1899) demonstrated that otoliths can be used as accurate indicators of both the age and size of fishes (see Meunier, 1988). These analyses utilize either the whole otoliths or sections (Pannella, 1971, 1974, 1980).

Otoliths begin as one or more central nuclei and they increase by concentric increments. Thin sections of otoliths show concentric annual bands called rings, zones or annuli which appear hyaline, transparent, translucent, dark or opaque (see ‘growth marks’in section 1V). The more commonly used terms are hyaline (= translucent) for the annuli and opaque (= dark) for the intervening zones, which correspond to slow-growth and fast-growth periods, respectively. Fine observations with acetate replicas or with SEM show fine daily growth rings which are useful for evaluating seasonality of growth in temperate and especially tropical fishes. Growth of otoliths takes place by daily increments, at least during the first years of life, as demonstrated by vitral labeling (Neilson and Geen, 1982; Schmidt, 1984; McFarlane and Beamish, 1987).

Seasonal environmental changes affect otolith calcification and growth rate through processes which are still unclear. An endogenous circadian cycle is entrained by the photoperiod, but this is also susceptible to modification by other cyclic variables (Campana and Neilson, 1985). In addition to annual and daily growth lines, otoliths commonly show bimonthly (fortnightly) and monthly growth patterns possibly related to lunar influence. These monthly bands provide a complementary basis for age determinations. Spawning rings are also microscopically distinguishable from winter rings in otoliths.

These techniques provide a means for archaeichthyologists to infer the season of death of fishes and, thus, to hypothesize the seasonality of human activities linked with fishing. In the field of palaeontology, fossil otoliths are used to estimate paleotemperatures by means of the 18O/16O ratios (see Casteel, 1976; Nolf, 1986). These results are commonly compared with data obtained from foraminifera or molluscs (Irie, 1960; Blacker, 1974; Campana and Neilson, 1985). An overview of otolith phylogeny among vertebrates has recently been attempted (Maisey, 1987).

VII. Evolutionary Trends

As evident from the preceding sections, fossilized skeletal tissues offer the exciting possibility of studying vertebrate evolution not only at the classical level of gross anatomy but also at the tissue level, as recognized by Enlow and Brown (1956-58).

The mineralized dermoskeleton, formed of dental tissues and bone, appears to be phylogenetically older than the mineralized components of the endoskeleton, since it is well developed in early jawless fishes (Heterostraci) as old as the Late Cambrian. However, endoskeletal calcified cartilages are already well known in at least one Ordovician and many Silurian jawless vertebrates, e.g. in the Heterostraci (?) and Osteostraci as well as in later (Devonian) early jaw-bearing fishes (acanthodians, placoderms, chondrichthyans, etc.). Thus dermal and periosteal (perichondral) ossification evolved before true endochondral ossification, the early history of which is rather poorly known.

The earliest vertebrates (‘fishes’ sensu lato) are characterized by extensive differentiation and variability of their dermoskeletal dental tissues. Consequently, research on these ancient fossils focuses on these tissues, rather than on bone and cartilage. The study of these dermoskeletal elements is important for elucidating several general problems such as the origin of the vertebrate skeletal tissues, the geological time of appearance of the various tissues, and the ontogenetic and phylogenetic relationships among the tissues. Since the pioneering works of the nineteenth century, pre-eminent studies in this field include, among many others. Goodrich (1907), Gross (1930, 1935, 1956, 1961, 1966, 1967, 1971). Denison (1963), Halstead-Tarlo (1964, 1965), Halstead (1969, 1973, 1974), Peyer (1968), Schmidt and Keil (1971), Schultzz (1969), Smith (1984, 1985), Smith et al. (1987, and notably Ørvig (1951, 1965, 1976a,b, 1978a,b,c, 1985), who has made outstanding contributions.

In comparison with fishes, tetrapod paleohistology and comparative histology are more devoted to the study of bone tissue itself, and to a far lesser extent cartilage. The dental histology of tetrapods is not as diverse structurally as among the fishes, and this is confined to the teeth themselves. Outstanding comparative histological and paleohistological surveys of tetrapod skeletal tissues have been offered by Seitz (1907), Foote (1916), Gross (1934), Ampnro and Godina (1947), Enlow and Brown (1955-58), Schmidt and Keil (1971), Enlow (1969) and Miles (1967). Comparative studies of epiphyses, their growth mechanism and endochondral bone have been most ably reviewed by Haines (1942, 1969). These comparative works form the factual and conceptual framework for current studies in this field.

The following is an overview of the main findings of vertebrate paleohistology with emphasis on bone tissue itself. This is followed by a general survey of the evolutionary consequences of these data.

A. Enamel and Ganoine

Enamel is restricted to teeth in all tetrapods but it is distributed on all tooth-like structures and scales in ‘fishes’. This tissue is relatively well studied among mammals which show extensive histological variations with both functional and systematic significance. Non-mammalian enamel is currently described as ‘non-prismatic’ or ‘pseudo-prismatic’ in most cases, although recent investigations among living and fossil reptiles clearly show that true prismatic enamel is probably much more widespread than previously suspected. ‘True’ (epidermal) enamel is known among living amphibians (at least after metamorphosis), sarcopterygian bony fishes and, most likely, among chondrichthyan teeth and scales as well. Among actinopterygian bony fishes, the situation is made complex by the common development in the teeth of hard superficial varieties of dentine, such as durodentine, which is sometimes interpreted as ‘true’ enamel (see below). However, in many cases, durodentine and true enamel appear to be associated in the same tooth (see discussion of acrodentine by Ørvig (1978a,b,c). The ganoine of actinopterygian scales has also been recently shown to have an epidermal origin, and it is thus homologous to tooth enamel (Sire et al., 1987). Differences, however, include a stratified structure in ganoine and a permanent position below the overlying epidermis, in contrast with the generally non-stratified structure and exposure of dental enamel, free from the epithelium in mature teeth. For these reasons, ‘ganoine’ should be retained in its usual descriptive sense,
with the understanding that it is very closely related in structure and development to dental enamel.

B. Cosmine

Cosmine is not a tissue, but rather a specialized histological complex which, in the scales and dermal bones of some early fishes, consists of a thin enamel-like superficial component with underlying dentine and a specialized system of ampullae opening at the surface by minute pores and which, very likely, housed specialized sensory cells (Thompson, 1975; Meineke and Thomson, 1983; Meineke, 1984). As no living sarcopterygian fish retains this peculiar specialization of the superficial dermoskeleton, it is uncertain if the superficial component here was indeed a true (epidermal) enamel, although this appears very likely.

C. Dentine

Although generally well characterized as a centripetal deposition around a pulp cavity, by Tomes' fibers, and by its superficial location close to the epidermis (below the enamel), dentine is a structurally variable tissue, especially among lower vertebrates. Dentine tends to show a higher polarization of its forming cells, or odontoblasts, in the more advanced lineages. The mesodentine and semidentine of the Osteostracii and placoderms, respectively, still have cell bodies trapped within the calcified extracellular matrix, as in bone. However, the more advanced orthodentine found in most fossil and living vertebrates excludes cell bodies from the calcifying matrix; they leave behind only fine, long, cytoplasmic processes forming the typical 'Tomes' fibers, which may even be lacking in some highly evolved dentines. Dentines commonly show diverse and complex histological organizations, such as vasodentines (or vascular dentines), osteodentines, trabecular dentines and plecidentines, the last one forming the labyrinthine teeth of crossopterygians, stegocephalians, etc. (Schultze, 1969). Superficial specializations of dentine by hypermineralization are also common (vitrodentines, durodentines, etc.) among lower vertebrates. While detailed, synthetic surveys and assessments of such 'enameloids' or 'mesodermal enamels' in connection with dentine proper are available (e.g. Ørvig in Miles, 1967; Smith, 1983-1984), many additional studies with new techniques are still needed to decipher the developmental and comparative-phylogenetic problems related to these tissues.

D. Pleromic Hard Tissues

As with dentine, additional histological, developmental, biochemical and mineralogical studies are necessary before the phylogenetic significance of pleromic hard tissues in several group of fishes can be fully understood (see Ørvig in Miles, 1967; Ørvig, 1976a; Smith, 1984, 1985; Smith et al., 1987; Smith and Campbell, 1987).

E. Cementum

Cementum has a rather poorly known evolutionary history. Cementum-like tissues occur in certain osteichthyan where they attach pleurodont teeth to dentigerous bones. Root cementum appears in various lineages with thecdodont tooth insertion, i.e. with a deep root which is suspended by a desmodont to the wall of true alveoli, a system which provides a shock-absorbing function as well as tooth drift after ankylosis. This occurs in mammals, some mammal-like reptiles (Osborn, 1984), archosaurian reptiles and early birds. Coronal cementum, on the other hand, appears to have a relatively late evolutionary appearance and is known only in true mammals. The structure of cementum is generally very close to that of bone, with, however, important variations in mineralization. It may contain cells (cementocytes), sometimes weak vascularization, and may even show resorption and secondary reconstruction. 'Growth rings' are especially noteworthy in this tissue, and they have very interesting skeletotchronological applications in mammals.

F. Bone and Plywood-like Structures

Bone shows a very wide spectrum of structural variations among vertebrates, since most of its usual components (including cells, blood canals, extrinsic fibers and minerals) are lacking in some specialized situations. Asperin, the early form of acellular bone found in the dermal skeleton of the earliest (Cambrian and Ordovician) vertebrates, the jawless Heterostraci, may not be ancestral to cellular bone, because the latter is known in fossils of unknown degree of Middle Ordovician age (Ørvig, 1965).

Plywood-like structures, sometimes extremely well developed in the dermal skeleton of living and fossil lower vertebrates (where they form the so-called isopodin of scale basal plates) are, in spite of obvious structural peculiarities, closely akin to bone. Plywood-like structures may be acellular, avascular, or they may partly or entirely lack mineralization, but they are clearly recognizable by their highly ordered and exceptionally large collagenous fibrils or bundles. On the other hand, comparative and developmental studies (Meunier, 1980b, 1984) demonstrate their basic agreement with bone, and recent works (Giraud-Guille, 1988) even show that the basic 'lamellar' structure of bone osteons also agrees with dermal 'plywoods'. In both cases, cholesteric liquid crystals offer excellent physical models to decipher the spatial organization of the fibers (Boulgand, 1978).

The most important studies in comparative histology of bone (Foote, 1916; Amptino and Godina, 1947; Enlow and Brown, 1956-58) have all clearly stressed the high diversity of bone histological patterns among vertebrates. This clearly contradicts the standard 'textbook' description of bone as a tissue, which is always based on standard biomedical materials (human, rat, mouse, etc.). This great diversity raises some doubts about the overall validity of many generalizations of bone biology and physiology derived from taxonomically restricted materials. Conversely, the analysis of this histological diversity may well lead to important functional and phylogenetic interpretations. Vertebrates differing in phylogenetic position, size, longevity, adaptive trends, ecology, etc., should show some reflection of these differences in their bone histology.

If we restrict our survey to the histological structures of bone shafts among living bony vertebrates, certain generalizations may be summarized as follows. Among bony fishes, compact bone tissue may be true lamellar, woven or parallel-fibred, and sometimes acellular or avascular, with extensive 'growth rings'. Haversian remodeling is generally weak, except among some large forms with a very active metabolism (tetras). Living
amphibians have generally simple compact appositional bone tissues, lamellar or pseudolamellar, often poorly vascularized and with obvious growth rings. Among reptiles, a rather similar condition prevails in the Squamata (lizards and snakes) which show, with few exceptions such as varanids, a mostly avascular, parallel-fibred or lamellar primary bone. Chelonians (turtles and tortoises) and crocodilians, on the other hand, have much more complex appositional bone tissues, often highly vascular, and in larger, older individuals more or less reconstructed by Haversian substitution in its most internal parts. Birds show generally highly vascularized and complex primary fibro-lamellar appositional bone tissue, with rather intense Haversian replacement. Mammals show highly diverse conditions in their bone compacta. Generally, mid-sized to large or very large herbivorous mammals show densely vascularized fibro-lamellar primary bone, where the vascular canals often form a highly-ordered plexus (e.g., plexiform tissue), to some extent replaced later by Haversian bone. Carnivores have much more diverse primary (appositional) vascular bone and can also show extensive Haversian substitution. Large primates with great longevity (humans) have, in older individuals, dense Haversian bone tissue in most parts of the cortex. Small primates, rodents and insectivores may show much simpler primary bone patterns with moderate Haversian substitution with remodeling (erosion and reconstruction) most active in the cancellous regions. Sea-living mammals may exhibit several bone histological specializations. For instance, among cetaceans, some parts of the skeleton, such as the flippers, may lack true compact bone tissue, while sirensians (manatees) exhibit spectacular pachyostosis in the ribs and other parts of the skeleton.

The compact bone tissues of most living poikilothermic vertebrates generally show a succession of conspicuous annual ‘growth lines’ consisting of a wide zone of active growth and a narrower annulus (Peabody, 1961), or even a line of arrested growth, with overall incomplete Haversian substitution. Homeotherms may also show bone growth lines, especially in the dentary bone but, with the exception of sea-going species, the skeletocochronological value of the lines is open to question, as the primary bone tissue is often densely vascular and Haversian substitution becomes extensive, especially among large, long-lived species. All the general histological differences recalled above are apparently related to the overall systematic position of the organisms as modified, of course, by ontogenic differences in bone histology linked with the local developmental conditions.

The comparative study of compact bone tissues among living vertebrates might lead to the conclusion that it has proceeded from simpler to progressively more complex and elaborate osteological structures from fishes to mammals (Crawford, 1940). Paleohistological data demonstrate, however, that this once-accepted simple scheme of regular evolutionary increase in complexity is incorrect. As soon as early tetrapods appear in the fossil record (Upper Devonian and Carboniferous), they already possessed (as did the ‘fishes’ from which they evolved), highly complex and diverse bone tissues. In fact, the archaic early amphibians (stegocephalians) had much more complex bone tissues than living representatives of the Amphibia. Thus, the latter appear to be secondarily simplified. Similar situations arise among most fossil reptiles of the Paleozoic and Mesozoic, which often had highly complex appositional bone tissues, rather similar to those of stegocephalians, much more diverse and highly organized than those of lizards and snakes, the predominant reptiles of today’s world. Among living reptiles, turtles and crocodiles show the most similar bone tissues to early tetrapods. Unlike most other reptiles, dinosaurs often had complex, well-vascularized fibro-lamellar primary bone tissues and extensive Haversian substitution similar to that among many large mammals and birds today. Grossly comparable functional situations in terms of rates of growth, longevity and perhaps thermal and metabolic physiology are probably related to those histological convergences. Fossil Cenozoic mammals do not differ significantly in bone histology from their living relatives. Even the mammalian predecessors, the advanced therapsids or mammal-like reptiles of the Late Permain and Early Triassic, already showed compact bone tissues similar to those in mammals.

G. Cartilages and Endochondral Ossification

Cartilage is a peculiar skeletal tissue because it can grow from within (intussusceptive growth) and it can expand as long as it has not become calcified. To this internal growth is added a peripheral apposition (normal accretionary growth) through the activity of a perichondrium. The earliest known calcified cartilages occur in the endoskeleton of *Eryphiocetus*, presumably an heterosarc in of Middle Ordovician age (Halstead, 1973; Ørvig, 1965). Its structure and globular (spheric) mineralization agree with the globular cartilages of modern tetrapods. Chondrichthyes have evolved highly specialized cartilages (e.g. areolar and prismatic; see Ørvig, 1951), but most other vertebrates retain globular cartilage with calcification. The cytoplasmic changes of cartilaginous tissue cells in the epiphyses of long bones are obviously linked to their growth mechanism. The cartilaginous epiphyses of early tetrapods may have initially originated in an ichthyohlast ancestor which did not already possess intra-epiphysal (secondary) ossification centers. On the other hand, the primitive pattern of tetrapod long bone epiphyses which characterizes stegocephalians, ‘cotylosaurs’, etc., is still expressed in some living vertebrates, such as chelonians and crocodilians.

The basic mechanisms of cartilage differentiation connected with longitudinal growth and endochondral ossification appear to have been retained, with fairly little change, among all tetrapods (Eggedahl, 1938).

The amount of cytological and tissue differentiation of cartilaginous zones (hyaline, seried, hypertrophied, etc.) in growing epiphyses, as well as their spatial distribution along the longitudinal axis, and, to a large extent, the spatial ‘order’ of the trabeculae of spongy bone which ultimately derive from the endochondral process of ossification, all appear to be related to the growth rate of the epiphysial region at a given stage during ontogeny, rather than reflecting taxonomic position. However certain taxonomic groups show distinctive and diagnostic relationships among their growth patterns and epiphyseal structures. Among urodeles, epiphyseal structures are commonly secondarily simplified compared with stegocephalians, especially among more or less paedomorphic taxa, where the process of endochondral ossification may be almost completely lacking. The resulting situation, whereby permanent calcified cartilages are retained to some extent within the ‘adult’ long bones, is also
commonly observed in various lineages of tetrapods secondarily adapted to aquatic habits. Hence, several stegocephalians, mesosaurs, nothosaurs, champsosaurs, and to some extent sirenians have been regarded as paedomorphic (Ricqlès, 1975b). Anurans have highly specialized "match head" epiphyses, possibly in connection with their jumping habits (Francillon, 1981). Calcification within epiphyseal cartilages, which might be regarded as independent secondary centers, occurs in anurans, lepidosaurus (Rhynchocephalia: Sphenodon), lizards, birds and mammals, but this is lacking in urodèles and Chelonian. True endochondral ossification of the secondary (epiphyseal) centers is well known at least among lizards and mammals, both lineages showing this development at least since the Jurassic. Among archosaurs, on the other hand, the situation is more complex. Apparently, crocodilians, thecodonts and dinosaurs have never had such secondary centers. Birds, however, are considered as having at least one true secondary center in the proximal head of the tibiotarsus, and many more examples have been tentatively described for other avian epiphyses. Despite such speculation on the evolution of secondary centers, neither their origin from independent sesamoids (or, conversely sesamoids derived from secondarily freed epiphyses), nor their origin via fusion with (and incorporation into) long bones of non-independent carpals and tarsals can generally be accepted now. It has been hypothesized that secondary centers evolved independently among several lineages, perhaps as specializations promoting finite growth in connection with ecological niches requiring small size. This may have happened during the Jurassic when large terrestrial animals consisted primarily of large archosaurs. Hence, lizards, early mammals, frogs and possibly birds would have become specialized for finite growth through differentiation of more or less similar secondary centers of ossification (Ricqlès, 1979). In summary, comparative and paleontological data on epiphyseal structures suggest that cartilage shows a rather stable structural pattern among vertebrates. However, various fine anatomical structures associated with cartilage development, such as cartilage canals, endochondral ossification and secondary centers, have evolved along specific lines in various independent lineages, sometimes expressing obvious examples of parallelism or convergence.

H. Concluding Remarks

Most authors recognize the great antiquity and overall evolutionary stability of vertebrate skeletal tissues, and thus of their underlying cytological and molecular mechanisms. Indeed, skeletal structures of the third and fourth orders, as defined by Petersen (1930) appear to have been established with the earliest known vertebrate fossils. Perhaps since the Late Cambrian, and certainly during the Ordovician, mineralized vertebrate tissues show the main characteristics of living forms, and some components appear to be already specialization in modern vertebrates. It thus seems clear that both the fundamental genetic-molecular mechanisms and the epigenetic constraints which produce the various specialized lineages of sclero blasts and their specific cell-products appeared very early during vertebrate phylogeny and have later experienced little, if any, change (Hall, 1973; Ricqlès, 1979). Mineralizable collagen fibrils, stellate scleroblasts, bone-trapped blood vessels, etc., have not changed their basic structure since the time of their initial appearance. A possible exception, however, is a shift from a prevailing ‘spheretic’ calcification in the earliest stage of skeletal tissue evolution towards the more advanced and complex ‘isotropic’ calcification of bone and most dentines (Orvig, 1968). It should be stressed, however, that this interesting hypothesis is still largely speculative. Hence, on balance, the evolutionary stability of the third and fourth order ‘elements’ of skeletal hard tissues appears to be a well-established fact.

But this point of view must be amended when we consider second order skeletal tissues which, in our analogy, represent the various ‘houses, castles and factories’ built from the same basic ‘bricks’. These tissues themselves are indeed integrated according to numerous variable combinations of the basic components of subordinate ranks already defined. The combinations can most often (but not always) be readily interpreted in terms of functional demands. Thus hard tissue structures appear to be clearly adaptive in most circumstances, rather than imposed by ‘phylogenetic constraints’ more or less independent of functional demands. This is not to say that some tissue peculiarities can never be used as ‘phylogenetic trade marks’ of specific taxa or lineages. However the rationale for using hard tissue characteristics in phylogenetic interpretations is often misunderstood (see Enlow, 1966, 1968; Ricqlès, 1975a, 1979). Generally, reliable taxonomic inferences from hard tissues samples of unknown origin, especially bone, can be obtained only if functional and ontogenetic interpretations are considered independently of a priori assumptions of taxonomic-phylogenetic relations. In most cases, at least with bone, histological variation imposed by local ontogeny so overwhelmingly exceeds variation which is reasonably directly linked with taxonomy that a taxonomic ‘diagnosis’ independent of ontogenetic factors appears, in most cases, to be hopeless (see Enlow and Ricqlès, 1975a, for further elaboration on this theme).

In summary, the history of bone and other vertebrate skeletal tissues can hardly be interpreted as demonstrating increasingly complex and irreversible evolutionary trends. Instead, this history shows ever-changing rearrangements of stable, basic components which evolved early in the history of the group (Enlow and Brown, 1958; Ricqlès, 1979). Vertebrate evolution is not characterized by the progressive appearance and refinement of new components of skeletal tissues. Indeed, the earliest vertebrates had already evolved the basic components of skeletal tissues, and later vertebrates primarily rearranged these components to form new combinations. The new combinations, on the other hand, appear to have varied but precise adaptive significance, with each combination representing an adaptation for new environments or new life habits. Like the skeletal tissues themselves, these new combinations do not appear in a single, linear, progressive pattern of increasing complexity, but in a multitude of patterns, each corresponding with particular adaptations. An especially obvious consequence of this process is the high incidence of evolutionary parallelism and convergence of bone tissues among distinct lineages with similar or analogous adaptations. From this point of view, it is meaningless to believe that dense Haversian bone, such as found in man, is the highest ‘evolutionary stage’ of bone tissue structure, as is commonly implicitly or explicitly assumed. This bone tissue pattern is merely associated with specific (if still poorly understood!) physiological circumstances dealing with growth, size,
mechanical demands, longevity, etc., which have been independently realized, time and time again, in many unrelated vertebrate lineages. There are clearly no 'primitive' or 'evolved' vertebrate hard tissues as such, but the histological arrangements of the basic components of these hard tissues precisely express the great adaptive trends successively or simultaneously expressed in various vertebrate lineages. Evolutionary changes at the tissue level may therefore be more properly described as a circumstantial 'history' rather than as evolution itself.

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