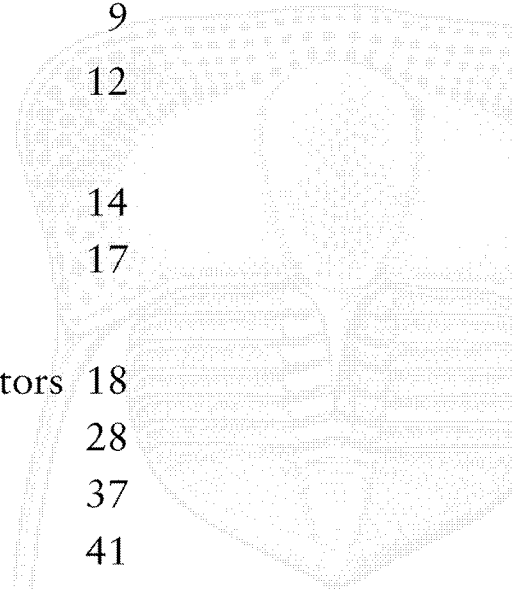


# The Palaeontology Newsletter

# 55

## Contents

Association Business	2
News	6
Association Meetings	9
Association Diary	12
Obituaries	
Leslie Rowsell Moore	14
Pierre Hupé	17
From our correspondents	
The hold of hypothetical ancestors	18
Palaeo-math 101	28
Acellular teleost bone	37
Stealing time	41
Advertisement: <i>Evolution of Pterosaurs</i>	47
The Mystery Fossil	48
Meeting Reports	50
Future meetings of other bodies	68
Book Reviews	78
<i>Palaeontology</i>	
vol 47 part1-3	93-95
Lyell Meeting abstracts	97



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## Acellular teleost bone: dead or alive, primitive or derived?

Amongst animal groups, rigid cellular skeletal structures are relatively uncommon, and may be restricted to the cartilages of non-vertebrates such as molluscs, arthropods and sabellid polychaetes (Cole and Hall, in prep.) as well as vertebrate cartilage and bone. However many animals have acellular skeletal tissues, for example molluscan shells, and annelid and arthropod cuticular exoskeletons. Even hemichordates have acellular skeletons derived from the hypertrophy of an epithelial basement membrane (Benito and Pardos, 1997). Our present column deals with a relatively unusual and yet intriguing instance where a cellular skeletal tissue appears to have secondarily evolved into an acellular skeletal tissue. The evolution of acellular bone is not merely a quirk restricted to a single group of organisms (teleost fishes), but as we shall see, has large-scale implications for the evolution of skeletal tissues in general.

The unlimited growth of bone in most vertebrates, its constant remodelling, and its response to mechanical and metabolic changes, define bone as a living tissue. Palaeontologists take advantage of information contained within the bone tissue in their structural analyses of fossilised skeletons to collect data about an organism's sexual maturity, age and growth rate (Castanet and Smirina 1990, Chinsamy 1995, Horner *et al.* 2000).

Bone as a tissue is characterized by three major cells types; osteoblasts, osteocytes and osteoclasts. Osteoblasts (bone-forming cells) are of mesenchymal origin, secrete non-mineralized bone matrix (osteoid), finally becoming entrapped in mineralized bone matrix as osteocytes. Osteocytes connect to other osteocytes and to osteoblasts on the bone surface via long cell processes. Osteocytes are considered to play an important role in strain detection and in the regulation of bone metabolism (Burger *et al.* 2003). Figures from human bone indicate that osteocytes constitute 95% of all bone cells and cover 94% of bone surfaces (Marotti 1996). Bone is resorbed by osteoclasts, which are macrophage-like cells derived from the haematopoietic cell lineage. In mammals and birds osteoclasts are typically multinucleated giant cells. Since regulation of osteoclast activity depends on osteoblasts and osteocytes, all three cell types together are regarded as a "basic multicellular unit" (BMU) (Frost, 1990). Finally, living bone tissue contains blood vessels, nerves and fat, and can accommodate haematopoietic tissue in late embryonic and postnatal tetrapods.

Taken together, bone is not just an inert mineralised hard structure, but a true metabolically active tissue. Nevertheless, in many (if not all) the osseous elements of the largest group of vertebrates, the advanced teleosts (Atherinomorpha, Paracanthopterygii, Acanthopterygii = Acanthomorpha, together representing approximately 16,000 species), the main cellular components of bone, the osteocytes, are missing. This phenomenon was first detected by Kölliker (1859) about 150 years ago and has since been described in detail. The osteocyte-lacking bone of advanced teleosts is called anosteocytic or acellular bone, in contrast to the osteocytic or cellular bone of tetrapods, basal teleosts, and primitive osteichthyans (Moss 1961; Meunier 1987; Meunier and Huysseune, 1992).



The lack of osteocytes has raised discussions whether acellular teleost bone is metabolically active, can be remodelled and ultimately mobilized for use as a mineral source. The reported lack, under normal conditions, of bone resorbing cells (osteoclasts) in teleosts with acellular bone (Glowacki *et al.* 1986) has further nursed the assumption that acellular bone is a “dead”, metabolically inactive, tissue. However, the presence of osteoclasts in acellular bone has been demonstrated (Sire *et al.* 1990), especially as essential elements during skeletal growth and tooth eruption (Witten and Villwock 1997, Witten *et al.* 1999). Furthermore, we now know that acellular bone can, at least under experimental conditions, participate in the animals’ mineral metabolism, even though phosphorous rather than calcium (the key factor in mammalian bone metabolism) seems to be the trigger for releasing minerals from the bone matrix (Witten 1997, Roy *et al.* 2004).

Osteoclasts involved in bone resorption in teleosts with acellular bone are usually mononucleated, and rarely create typical resorption lacunae (so-called smooth resorption) (Witten 1977). This is very different from typical bone resorption in mammals where multinucleated osteoclasts create deep resorption lacunae. The absence (or rarity) of multinucleated osteoclasts in teleosts with acellular bone may relate to the absence of osteocytes and haematopoietic bone marrow tissue, since both provide signals that are important for the formation and action of multinucleated osteoclasts (Burger *et al.* 2003). The inconspicuous appearance of osteoclasts in teleosts with acellular bone requires their detection through osteoclast specific enzymes (*e.g.*, tartrate resistant acid phosphatase, TRAP) or transmission electron microscopy (Witten *et al.* 2001, Sire and Huyseune 1993). Without such enzyme markers, proper identification of osteoclasts and sites of bone resorption in advanced teleost remains elusive (Witten 1997, Witten *et al.* 2001). Consequently, standard histological procedures alone do not provide the correct picture of resorption and bone remodelling in advanced teleosts.

The lack of easily recognizable indications for bone resorption and remodelling has important implications when it comes to the examination of fossilised bone structures from this group. Whereas in tetrapods and primitive bony fish, sites of bone resorption can be identified on the basis of typical resorption lacunae (as traces of the activity of multinucleated osteoclasts), this might not be the case for acellular bone in advanced teleosts. With this in mind it might be worth considering that structural analysis of fossilised skeletons from advanced teleosts does provide data about bone growth (commonly utilized elements include operculae, vertebrae, otoliths, fin rays) but that data about resorption and bone remodelling are likely to be overlooked or misinterpreted. A similar reason might also explain the lack of evidence for bone resorption in many groups of early gnathostomes (Donoghue and Sansom, 2002).

Apart from the debate about the degree to which acellular bone is metabolically active, there is a discussion about the first appearance of acellular bone in the course of vertebrate evolution (Ørvig 1964, Smith and Hall 1990).

With few exceptions (see Moss 1961, Meunier and Huyseune 1992), acellular bone in extant bony fish is restricted to advanced teleosts, which is a good reason to view acellular bone as a derived character within teleosts. On the other hand, acellular bone has also been reported from the base of chondrichthyan placoid scales (and teeth) and in the remarkable ‘spine brush’ of stethacanthids (Coates *et al.* 1998; Donoghue and Sansom 2002). This may indicate



that acellular bone did not evolve in advanced teleosts for the first time, suggesting that acellular, and not osteocytic, bone would be the primitive vertebrate bone type.

The occurrence of aspidine (considered to be a type of acellular bone) in the dermal skeleton of Heterostraci is often cited as an argument for acellular bone as the plesiomorphic condition (reviewed by Ørvig 1964 and by Smith and Hall 1990). Moreover, the simultaneous appearance of cellular bone in both the endoskeleton and dermal skeleton has been identified as a character of the stem group of gnathostomes (see Donoghue and Sansom 2002). Certainly, considerations of whether acellular bone is derived or primitive within vertebrates have to take into account whether one is dealing with elements of the dermal skeleton or of the endoskeleton, skeletal elements derived from neural crest or from trunk mesoderm, and/or intramembranous or endochondral modes of bone formation (see Palass Newsletters 53: 48–51 and 54: 17–21).

Comparative studies on bone development and structure in acellular- and cellular-boned teleosts might shed new light on the evolutionary origin of acellular bone in advanced teleosts. Indeed, in all instances examined, developing cellular bone in teleosts seems to go through a phase of acellularity, in which it much resembles acellular bone. Together with the sequence in which osteoclasts appear in cellular bone (first mononucleated, followed by multinucleated cells), these observations suggest that acellular bone might have arisen in advanced teleosts through a process of heterochrony (Witten *et al.* 2004).

The question then is how this interpretation could affect our view concerning the relation (developmentally, and/or evolutionary) of acellular bone to other acellular, or partly acellular skeletal tissues, like dentine, osteodentine, mesodentine, and aspidine (Smith and Hall 1990). Acellular teleost bone does not develop by cell processes retreating or by death of osteocytes (Ekanayake and Hall 1987, 1988). From a developmental perspective, there is thus no transitory tissue between acellular and cellular bone. Acellular bone contains no osteocytes (thus no lacunae) and no cell processes (Huyseune 2000); it is present both in the dermal skeleton and in the endoskeleton, it can be produced by both neural crest and trunk mesodermally derived mesenchyme (Hall 1978, 2004), and its appearance is not dependent on the presence of teeth. From an evolutionary perspective, these characters discriminate acellular bone from all other acellular skeletal tissues that have arisen in evolution. Basal plates of placoid scales/teeth, bone of attachment, and acellular cementum are dermal skeletal tissues that have arisen in relation to tooth/scale attachment and are restricted to the dermal skeleton.

In conclusion, acellular teleost bone is not static but an active tissue that is primarily remodelled by inconspicuous, mononucleated, bone-resorbing cells, without leaving easily identifiable traces such as deep resorption lacunae. Acellular bone appears to be a new feature of advanced teleosts that presumably evolved during the group's long evolutionary period in a stable and mineral rich marine environment.

**P. Eckhard Witten\***, **Ann Huyseune<sup>6</sup>**, **Tamara Franz-Odendaal**, **Tim Fedak**,  
**Matt Vickaryous**, **Alison Cole**, **Brian K. Hall**

*\*for correspondence on this column*

*The Hall lab and guest<sup>6</sup>, Department of Biology, Dalhousie University*

*<pwitten@dal.ca>*



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