Chapter One

THE STRUCTURE OF BONE TISSUE

THROUGHOUT this book I shall be suggesting that the structure of bone tissue, and of whole bones, makes sense only if its function, particularly its mechanical function, is known or guessed. (As Rik Huiskes of Eindhoven is fond of saying [2000]: "If bone is the answer, then what is the question?") However, in this first chapter I shall deal only with the structure of bone, leaving almost all discussion of function until later. Of course, the mechanical properties of bone and bones are determined by their structure, and we cannot begin to understand the function without having a good idea of the structure. Much of the subject matter will be familiar to some readers, but not all to everyone. Indeed, some readers may be coming to bone for the first time, from say materials science, so I shall start with a single paragraph overview of bone structure.

Bone of present-day mammals and birds is a stiff skeletal material made principally of the fibrous protein collagen, impregnated with a mineral closely resembling calcium phosphate. Bone also contains water, which is very important mechanically. Bone is produced inside the body and is usually covered with cells throughout life, though in fish scales, for instance, the external lining of cells may be rubbed off. Most bone not only is covered by cells but has living cells and blood vessels within it. Bone, being hard, cannot swell or shrink; all changes in shape must take place at surfaces. Most bones are hollow and contain hematopoietic or fatty marrow. Marrow probably has little mechanical significance. Tendons and ligaments insert into the bone substance, and the ends of bones are often covered by a thin layer of cartilage for lubrication. Some tissues, such as antler and dentin, are not called bone but are actually bone, or extremely like it. Horn, such as is found in cattle, is a completely different material, usually unmineralized, though the horn core, which supports the horn, is made of bone.

To start straight off talking about the structure of bone begs the question. It is not really at all clear what bone is. Consideration of a present-day mammal or bird would allow a clear distinction to be made, because bone is the only structure that is essentially collagen mineralized with calcium phosphate and containing cell bodies, though in antler bone the cells are all dead by the time the antler comes to be used. Dentin is collagen mineralized with calcium phosphate but it does not

contain cell bodies, only tubular extensions of cells. The other significant tissue mineralized with calcium phosphate (as opposed to calcium carbonate) is enamel, and this is very different in that it has virtually no cells or cell processes or, indeed, much organic matrix. However, as soon as one looks outside the mammals and birds the situation becomes much more complex. Bone is found only in vertebrates. Many teleost fish have bone without bone cells, and the range of structures seen in scales of different fish species forms an almost complete spectrum from what is obviously "typical" bone or "'typical" dentin to what is obviously "typical" enamel. This situation is often found in biology, since nature is concerned not with categorization, but with producing effective results. (The great evolutionary biologist John Maynard Smith likes saying that "all biology is false," meaning, of course, that there are very few absolutes in biology.)

For the purposes of this book, the fact that there are so many different types of scales does not matter greatly because virtually nothing is known about the mechanical properties of scales. I shall be concerned almost entirely with the mechanics of typical bone, as found in mammals, but I shall devote some space to tissues such as dentin. Antler, which is dead when functioning, will figure prominently. A good account of the variation of structure in vertebrates is found in Francillon-Vieillot et al. (1990), which, though not an enthralling read, is very clear, comprehensive, and well illustrated.

Even "typical" bone is such a complex structure that there is no level of organization at which one can truly be said to be looking at bone as such. I shall start at the lowest level and work up to a brief description of the variety of shapes one sees in whole bones.

1.1 Bone at the Molecular Level

At the lowest level bone can be considered to be a composite material consisting of a fibrous protein, collagen, stiffened by an extremely dense filling and surrounding of calcium phosphate crystals. There are other constituents, notably water, some ill-understood proteins and polysaccharides, and, in many types of bone, living cells and blood vessels. The amount of water present in bone is an important determinant of its mechanical behavior, and I shall say more about it in chapter 4.

A word about terminology. In this book I use the word *matrix* to mean the water and the soft organic material, mostly collagen, in which the mineral crystals are deposited. This accords with what materials scientists would consider to be the matrix (though some might consider the mineral to be the matrix). However, bone biologists, who are fo-

cused almost entirely on the cells of bone, use the word *matrix* to mean the bone tissue itself, that is, the water, the organic material, and the mineral. There is no way round this possible source of confusion; one simply has to be aware of it.

Collagen is a structural protein found in probably all metazoan animal phyla. It is the most abundant protein found in animals, but only in the vertebrates does it undergo a wholehearted transformation into a mineralized skeletal structure, although some soft corals have traveled some way along the road. A classified bibliography of more than 3400 references to collagen, comprehensive up to that time, is given in Kadler (1994).

Unmineralized collagen is also found in the vertebrates, and in many invertebrates, in skin, tendon, ligament, blood vessel walls, cartilage, basement membrane, and in connective tissue generally, in those circumstances where the material is required to be flexible but not very extensible. Collagen makes up more than half the protein in the human body (Miller 1984). Collagen from different sites often has different amino acid compositions; in the mid 1990s 19 types of collagen were known throughout the animal kingdom, and the known number increases relentlessly (Prockop and Kivirikko 1995). The collagens of skin, tendon, dentin, and bone share the same type of composition, and are called *type* 1 collagen. The protein molecule *tropocollagen*, which aggregates to form the microfibrils of collagen, consists of three polypeptides of the same length—two have the same amino acid composition, one a different one. These form on ribosomes, are connected by means of disulfide cysteine links, and leave the cell. Outside the cell the ends of the joined polypeptides are snipped off, the lost part containing the disulfide bonds. The three chains are by now held together by hydrogen bonds in a characteristic left-handed triple helix.

The primary structure of the polypeptides in the tropocollagen molecule is unusual, great stretches of it being repeats of glycine–X–Y, with X often being proline and Y sometimes hydroxyproline. The imino acids proline and hydroxyproline are unlike amino acids in that the nitrogen atom is included in the side chain as part of a five-membered ring. The effect of this is to reduce the amount of rotation possible between units of the polypeptide. It also prevents α-helix formation and limits hydrogen-bond formation. These constraints result in a rather inflexible polypeptide, 300 nm long (Olsen and Ninomiya 1993).

The tropocollagen molecules line up in files and bond, not with molecules in the same file, but with molecules in neighboring files, to form *microfibrils*. The tropocollagen molecules alongside each other are staggered by about one-fourth of their length. There is a gap between the head of one molecule and the tail of the next in the file, the *hole region*,

and, because many tropocollagen molecules are stacked side by side, these gaps and other features of the molecules produce a characteristic periodicity, 67 nm long. The whole microfibril becomes stabilized by intermolecular cross-links. Microfibrils aggregate to form *fibrils*. Although the longitudinal arrangement of the tropocollagen molecules in the microfibrils is fairly well understood, the way in which the microfibrils themselves are arranged laterally to form fibrils is much less well understood. A clear introduction to the subject is provided by Prockop and Fertala (1998). Hulmes et al. (1995) produce evidence that the fibrils are arranged in concentric rings. Wess et al. (1998a,b) produce a rather different model that they claim will explain the way in which mineral is able to pack in bone. This is a difficult subject, with the majority view changing often as experimentation becomes ever more sophisticated. It could well be that when a stable view is formed the results will be useful in helping to model the mechanical behavior of bone, but at the moment this is not really the case.

Collagen comprises about 85 to 90% of the protein in bone. The proteins that are not collagen are called, negatively, *noncollagenous proteins* (NCPs). The literature on them is vast and expanding rapidly (Ganss et al. 1999; Gerstenfield 1999; Gorski 1998; Nanci 1999). Some NCPs are restricted to bone, and some are also found in other places in the body. Some of these proteins almost certainly have a role in the initiation and control of mineralization or reconstruction, and some may have a role in binding the collagen and mineral together (Roach 1994). However, we are almost completely in the dark at the moment about any quantitative effect NCPs may have on the mechanical properties of bone.

Impregnating and surrounding the collagen is the bone mineral, which is some variety of calcium phosphate. The precise nature of the mineral of bone, both its chemistry and its morphology, is still a matter of some dispute. The problem is that the mineral in bone comes in very small crystals that have a very high surface-area-to-volume ratio. The size of the crystal is such that in one dimension it is only about 10 atomic layers thick (Lowenstam and Weiner 1989). This makes it reactive, and so most preparative techniques used for investigating it, such as, drying under vacuum for electron microscopy, may cause alterations from the living state. There is agreement that some of the bone mineral is the version of calcium phosphate called hydroxyapatite, whose unit cell (the smallest part of a crystal that is repeated uniformly throughout a crystal) contains $Ca_{10}(PO_4)_6(OH)_2$. The crystals are impure. In particular, there is about 4–6% of carbonate replacing the phosphate groups, making the mineral more truly a carbonate apatite (dahllite). This carbonate substitution takes place more near edges of the bone, close to

vascular and marrow spaces and tends to reduce the crystallinity of the crystals (Ou-Yang et al. 2001). Various other substitutions may take place (Boyde and Jones 1998; McConnell 1962).

At the moment, we are ignorant of the mechanical properties of the mineral itself, and all modeling, such as that of Wagner and Weiner (1992) and Sasaki et al. (1991), which I discuss in section 3.7, makes use of somewhat insecurely based (though not necessarily far wrong!) estimates. The mineral is certainly stiff, but its strength, in such small blocks, is unknown.

The positioning of the mineral relative to the collagen fibrils, as well as its shape, is becoming clearer, though there is still controversy. There is some argument as to whether the crystalline mineral, which can be seen in electron micrographs, is needle-shaped or plate-shaped. Ascenzi et al. (1978) claimed that the mineralization process starts off with small granules, about 4.5 nm across, which coalesce or grow into needles about 40 nm long. However, the observations of Landis and his coworkers make it almost certain that in mineralized tendon (Landis et al. 1993) and in embryonic chick bone (Landis et al. 1996) the crystals are platelet-shaped. They have used the technique of taking multiple views of bone using high-voltage electron microscopy to produce a tomographic image. This method shows very clearly the three-dimensional shape of the crystals and to some extent their spatial relationship to the collagen (fig. 1.1). These visualizations show that the crystals' thickness is rather unvarying at about 4–6 nm, their width is about 30–45 nm, and their length is typically 100 nm. Later, these mineral platelets seem to fuse sideways, and lengthways, producing at times sword-shaped blades that are quite long and broad. However, they do not seem to grow in the depth direction, remaining about 5 nm deep. Erts et al. (1994), using scanning probe microscopy, found similar values for turkey tendon.

Reports of the visualization of the crystals directly overwhelmingly supports this view that the crystals in all bone examined are platelet-shaped. Weiner and Price (1986) examined the size of bone mineral crystals, extracting them from the bone by a gentle procedure, and proposed values of about $50 \times 20 \times 2$ nm. Kim et al. (1995) report platelet-shaped crystals from tissues of a taxonomically satisfyingly varied group of species: chickens, bovines, mice, and herring. The average length and breadth, in nanometers, for the four species are given in Table 1.1. Kim et al. did not measure the thickness, but suggested it was about 2 nm. Ziv and Weiner (1994) suggest that most estimates of the size of crystals are underestimates, because the plates are so fragile, and that crystals may be often hundreds of nanometers long in untreated bone.

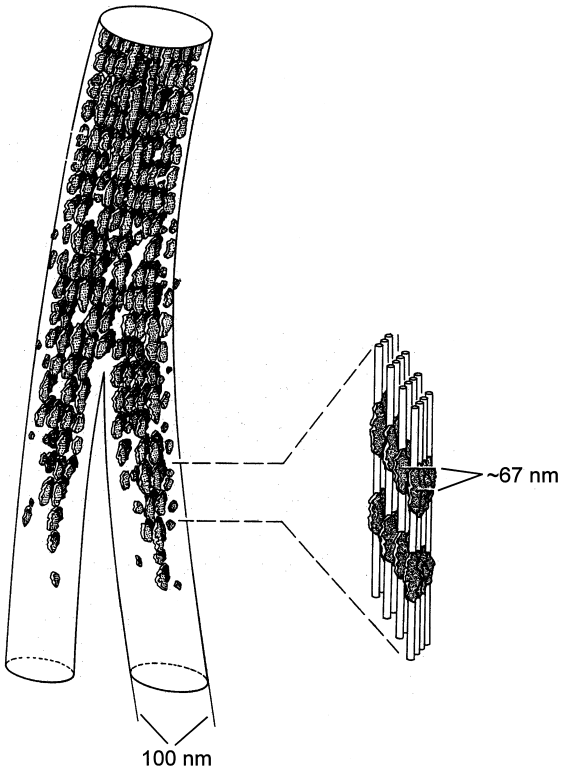

FIG. 1.1 Diagram of mineralizing turkey leg tendon according to the microtomographic investigations of Landis. Mineralization is proceeding from the top down. The crystals are platelet-shaped, and are initially registered in line with the hole region of the fibrils (~67 nm apart). This initial relationship between the platelets and the collagen fibrils is shown in the right-hand part of the diagram. Toward the top of the diagram some platelets are shown as having fused longitudinally. (From Landis, W. J., Hodgens, K. J., Arena, J., Song, M. J., and McEwen, B. F. 1996. Structural relations between collagen and mineral in bone as determined by high voltage electron microscopic tomography. *Microscopy research and technique* **33**:192–202. Reprinted by permission of Wiley–Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Fratzl et al. (1992) have produced indirect evidence, using small-angle X-ray scattering, that the crystals in ossified tendon are indeed platelet-shaped but that in ordinary compact bone they are more likely to be needle-shaped. On the other hand, Wachtel and Weiner (1994) show that the small-angle X-ray scattering picture from crystals from rat bone is very similar to that from mineralizing turkey tendon, and suggest that it is probably reasonably safe to generalize about the crystal morphology from mineralizing turkey tendon.

TABLE 1.1
Length and Breadth of Mineral Crystals (in nm) in the Bone of Four Species

Species	*Length*	*Breadth*
Chicken	23.3	12.2
Bovine	27.3	15.8
Mouse	21.2	12.0
Herring	37.3	15.4

Source. From Kim et al. (1995).

More contentious is where the mineral is in relation to the collagen fibrils. For years, following a suggestion by Hodge and Petruska (1963), it was thought that the mineral is initially deposited in the holes between the heads and the tails of the tropocollagen molecules (the gap zones). This results in the initial mineralization having a 67-nm periodicity (Berthet-Colominas et al. 1979). Many studies seemed to confirm this, but nearly all were carried out on mineralizing turkey leg tendon, which, although very convenient to study because the collagen fibrils are so well arranged, is not typical bone, particularly in relation to the arrangement of the crystals (Wenk and Heidelbach 1999). It is probable that in some way the particular conformation of the collagen molecule allows it to act as a nucleation site, permitting the precipitation of lumps of mineral that, without the presence of the energetically favorable sites, could not come out of solution. There is some evidence that the mineral deposits preferentially in parts of the fibril that are high in hydrophilic residues (Maitland and Arsenault 1991). Later, the mineral is deposited all over the collagen fibrils, and also within them. Weiner and Traub (1986) have published stereopairs of mineralizing turkey leg tendon, showing how the crystals lie within the fibers. Landis et al. (1993, 1996) show similar pictures (fig. 1.1), and point out that the individual platelets seem to remain separated by a space in the depth direction of about 5 nm. This would, of course, allow collagen microfibrils to exist between the platelets. Jäger and Fratzl (2000) suggest, though with no observations to back the suggestion up, that the crystals may be arranged circumferentially round the center of the fibril. This would accord with the radial fibril model of Hulmes et al. (1995).

All these observations relate to early stages of mineralization, and it is much less obvious what happens when mineralization has proceeded to its full extent. There must be considerable derangement of the initially very uniform collagen structure. It is strange that some quite modern works (e.g., An [2000], table 3.1) still hold to the idea that the only place that the mineral resides in mature bone is in the gap

region between the ends of adjoining collagen molecules. This idea is quite wrong.

Much work has been done on the mineralizing turkey leg tendon, because, although it is called tendon, it is actually proper bone and mineralizes in a particularly regular way, so that there is a close relationship along the tendon between distance and the progression of mineralization. This makes it very convenient for studies of mineralization. However, most bone mineralizes on surfaces, rather than at the end of tendons. On surfaces what happens is that a matrix of collagen, plus a few other organic components, is laid down first. This organic material is called *osteoid*. Mineral is deposited in the collagen, initially, it seems, in the gap zones, but then, unlike the situation in tendon, all along the length of the collagen fibrils. The plate like mineral crystals as they grow tend to form quite large lumps, and the individual mineral crystals tend to be oriented in the direction of the collagen fibrils. Within the collagen fibrils the precipitation is not random; one of the long axes of the mineral plates is always fairly well aligned with the collagen fibrils. Also, later, mineral is deposited between the fibrils, in the amorphous and rather tenuous ground substance. The relative amount deposited within and between the collagen is quite hotly argued even now, with some people proposing that more is between than within (Bonar et al. 1985; Lees et al. 1990; Pidaparti et al. 1996). Furthermore, it is likely, certainly in mineralized tendon and possibly ordinary bone, that the mineral outside the fibrils may have a different orientation from that within the fibrils (Lees et al. 1994; Pidaparti et al. 1996). In some fish bone, however, the vast majority of the mineral is within the fibrils, and not between them (Lee and Glimcher 1991). Knowing the truth of these matters is important for understanding why bone behaves mechanically as it does.

Added to our ignorance of the disposition of the mineral in bone is our ignorance of how, and the extent to which, the collagen and the mineral are bound together. The relationship between those mineral crystals that are inside the collagen fibrils and the collagen is extremely intimate, and such short-range forces as van der Waals forces may well be important. Also, ionic bonding probably occurs, which I discuss in section 3.7.

I am conscious, in reviewing the last few pages, of how often I have said that we are ignorant of the true situation. I suspect that much of our ignorance will be removed in the next decade. It will then become possible to try to understand in detail how collagen/mineral interactions determine the mechanical properties of bone at the molecular level, in the same way that metallurgists have a good idea of how, for instance, steel behaves at this level. However, bone has several levels of structural

hierarchy above the molecular level, and these all have important effects on the mechanics of bone, as we shall see.

1.2 The Cells of Bone

Bone is permeated by and lined by various kinds of specialized cells, which will be introduced later. I here list them and briefly describe their properties.

Bone-lining cells cover all surfaces of bones, including the blood channels, forming a thin continuous sheet that controls the movement of ions between the body and the bone (Miller et al. 1988). The layer of cells on the outside of the bone is called the *periosteum*, although this word is often used to include the strong collagenous sheet covering the outer surface. The layer of cells on the inside of the bone is called the *endosteum*. The bone-lining cells, which are often considered to be quiescent osteoblasts, are derived, via complex series of changes, from osteoprogenitor cells. These stages are described by Lian and Stein (1996).

Osteoblasts derive from bone-lining cells and are responsible for the formation of bone. They initially lay down the collagenous matrix, *osteoid*, in which mineral is later deposited, and they probably also have a role in its mineralization.

Osteocytes are the cells in the body of the bone. In cancellous bone the density of osteocytes varies from about 90,000 mm^{-3} in rats to about 30,000 mm^{-3} in cows. In general, the larger the animal the lower the density of osteocytes (Mullender et al. 1996). They derive from osteoblasts. They are imprisoned in the hard bone tissue and connect with neighboring osteocytes and with bone-lining cells by means of processes that are housed in little channels (canaliculi), of about 0.2–.03 μm diameter (Cooper et al. 1966). The actual connections with neighboring cells are by means of *gap junctions* that allow small molecules through easily.

Osteoclasts are bone-destroying cells. They are large, multinucleated cells derived from precursor cells circulating in the blood. In time-lapse photography they give the appearance of being extremely aggressive, clamping themselves to the bone's surface and leaving a space underneath a *ruffled border* that is very mobile and beneath which the bone can be seen dissolving. Debris, both organic and mineral, are packed into little vesicles and pass through the cell body of the osteoclast and are dumped into the space above (Nesbitt and Morton 1997; Salo et al. 1997). When osteoclasts have done their job they disappear and presumably die. (A colleague of mine, who initially studied osteoclasts, said that for some strange reason most people who studied them were as

aggressive as their subjects. My colleague eventually gave up, and turned to studying benign osteoblasts!)

1.3 Woven and Lamellar Bone

Above the level of the collagen fibril and its associated mineral, mammalian bone exists in two usually fairly distinct forms: woven bone and lamellar bone. Parallel-fibered bone is intermediate.

Woven bone is usually laid down very quickly, more than 4 μm a day and often much more, most characteristically in the fetus and in the callus that is produced during fracture repair. The collagen in woven bone is variable, the fibrils being 0.1–3 μm or so in diameter and oriented almost randomly, so it is difficult to make out any preferred direction over distances greater than about a millimeter (Boyde 1980; Boyde and Jones 1998). The mineralization process involves roughly spherical centers, impregnating both the collagen and ground substance at the same time, in which the crystals seem to be randomly arranged. As these mineralization centers spread they abut and often leave mineral-free spaces (Boyde 1980). As a result, woven bone, though highly mineralized, is often quite porous at the micron level. As in most bone, woven bone contains cells (osteocytes) and blood vessels. Rather frequently, the spaces surrounding the osteocytes are extensive and differ in this way from those in lamellar bone. There are of the order of 60 canaliculi per osteocyte (Boyde 1972), though no doubt this number varies greatly between osteocytes and between species. "Woven" bone is a misnomer, because there are very few examples of weaving, that is, true interlacing, in biology. (It would be a trick almost impossible to bring off, though, surprisingly, the enamel of some rodents has something almost as good—structures arranged in three orthogonal directions, see Section 6.3.)

Lamellar bone is more precisely arranged, and is laid down much more slowly than woven bone, less than 1 μm a day (Boyde 1980). The collagen fibrils and their associated mineral are arranged in sheets (lamellae), which often appear to alternate in thickness. The final degree of mineralization of lamellar bone is less than that of woven bone. The classical view is that the fibrils lie within the plane of the lamella, rarely passing from one to the next and that the fibrils tend to be oriented in one direction within the lamella. Indeed, some workers suggest that the collagen fibrils in a particular lamella are all oriented in the same direction (Ascenzi et al. 1978). However, this is probably not the case; in many lamellae the fibrils are in small *domains* about 30–100 μm across. Within a domain the fibril orientation is constant, but it changes, within

one lamella, from one domain to the next (Boyde and Hobdell 1969; Frasca et al. 1977). The collagen fibrils in lamellar bone form branching bundles, 2–3 μm in diameter (Boyde 1980), thicker than in most woven bone. The osteocyte lacunae in lamellar bone are oblate spheroids, the equatorial diameters being about five times longer than the polar axis. The shorter axis of each lacuna is oriented parallel to the direction of the thickness of the lamella.

The division between one lamella and the next looks abrupt under the light microscope, particularly under polarized light. However, scanning electron microscope pictures show a much messier situation. Frequently, lamellae *seem* to come in alternating thicknesses. There is a relatively thick one, about 5 μm thick, whose mineral crystals are roughly arranged all in one direction, over small distances at least, at an angle both to the long axis of the bone and to the circumferential extension of the lamella itself The thin lamella is about 1 μm thick, and has mineral plates oriented in the plane of the circumferential extension of the lamellae, with their smallest dimension normal to the long axis of the bone. This kind of lamellar organization is held to occur in mice by Weiner et al. (1991), and from my observations of fracture surfaces of bone of a large number of species I think this arrangement is widespread. If the mineral is arranged like this, presumably the collagen fibrils also lie in the same direction. As we shall see in the next chapter, such changes in direction have mechanical consequences. There is disagreement at the moment as to whether there is a difference in the density of mineral in the thick and thin lamellae. Marotti (1993) argues, giving evidence from the appearance of the collagen fibrils, that the thicker lamellae are looser and have more mineral, the thinner lamellae are somewhat more collagen-rich, and there is not a great deal of difference in the collagen orientation between the different layers. Yamomoto et al. (2000) argue convicingly that, at least in human dentin, the appearance found by Marotti is an artifact and that the differently appearing lamellae are only different in respect of their orientation. The uncertainty in the literature may be partially because some people concentrate on the mineral and some on the collagen.

Giraud-Guille (1988) produces strong evidence that in some lamellae the collagen fibrils, and presumably, therefore, their associated mineral crystals, are arranged in a "twisted plywood" or helicoidal structure. In a helicoidal structure there are many layers, and within a layer the fibers all point in the same direction. There is a change of angle between the layers, usually quite small, and this is constant in size and sense. The result is a multilayer composite with no preferred orientation. Ziv et al. (1996), Weiner et al. (1997), and Weiner and Wagner (1998) produce further evidence that such continuous transitions between, and even

through, apparently discontinuous lamellae are common. The model of Weiner and his co-workers suggests that collagen fibrils, only 80 nm or so in diameter, are the basic unit. These may lie parallel to each other through the thickness of the lamella, or be at an angle, usually about 30°, to their neighbors. Helicoidal arrangements of fibers are found frequently in biological tissues, and Neville (1993) has written a whole book about them. They occur in all sorts of structures, such as insect cuticle and plant cell walls, and are a prime example of the ability of organisms to produce complex structures by self-assembly, that is to say, without cells being involved directly in the placing of the succeeding layers. Helicoidal structures give rise to a confusing artifact if cut and examined at an angle to the plane of the sheets: The structure appears to consist of a series of arcs (Kingsmill et al. 1998, fig. 5f), but, in fact, there are no arcs.

Parallel-fibered bone (described by Ascenzi et al. [1967] and by Enlow [1969]) is structurally intermediate between woven bone and lamellar bone. It is quite highly calcified, but the collagen fiber bundles are much more parallel than those in woven bone.

1.4 Fibrolamellar and Haversian Bone

In mammals there are, at higher levels of structure, four main types of bone. Woven bone can extend uniformly for many millimeters in all directions. Such a large block is found only in very young bone (of rather large mammals) and in large fracture calluses. Lamellar bone may also occupy quite large volumes. Usually, in mammals it does so in circumferential lamellae, initially wrapped around the outside or the inner cavity of bones. There are blood channels in such bone, but they do not much disturb the general arrangement of the lamellae.

Lamellar bone also exists in a quite separate form: Haversian systems, or secondary osteons. The British and other Europeans are inclined to use "Haversian systems," whereas Americans prefer "secondary osteons." It does not matter which is used, except that it is critical to distinguish primary osteons, which I shall describe in a moment, from secondary osteons. Haversian systems form like this: many bone-destroying cells, called osteoclasts, move forward in a concerted attack on the bone tissue. They form a so-called *cutting cone*, shaped, as Martin and Burr (1989) say in an excellent account of the process, like "half an eggshell which is about 200 μm in diameter 300 μm long." Osteoclasts are not derived from cells that occur locally, but instead come from cells circulating in the blood. As the cutting cone advances it leaves a cylindrical cavity of diameter about 200 μm behind. Almost as

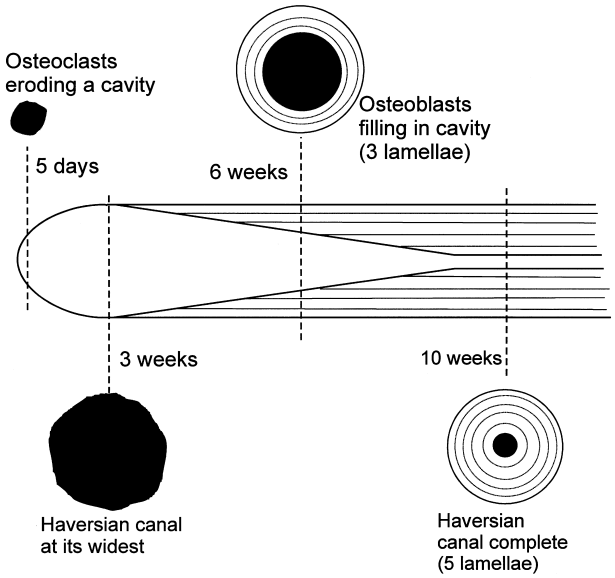

FIG. 1.2 Diagram of a forming secondary osteon (Haversian system) in longitudinal and cross-sectional views. The system is extending toward the left. The times give, very roughly, the time course of the process in humans. At 5 days the osteoclasts are still widening the cavity in the bone. At 3 weeks the cavity it at its widest. By 6 weeks the cavity is half filled in by osteoblasts, and by 10 weeks or so the process is completed, although it will take a long time for the bone to become completely mineralized. In the cross sections the central cavity is shown black.

soon as the cavity forms, it begins to fill in (Parfitt 1994). The walls of the cavity are made smooth, and bone is deposited on the internal surface in concentric lamellae (fig. 1.2). The end result is arranged like a leek *Allium porrum*, with usually clearly distinguishable cylindrical layers, except that there is a central cavity in the Haversian system, which contains one, or sometimes two, blood vessels, and nerves (Marotti and Zallone 1980). In humans the whole process from initiation of the osteoclastic activity to the completion of the filling in takes about 2–4 months.

The Haversian system is the classic result of the process of remodeling. There are two ways in which new bone may appear. In *modeling* the gross shape of the bone may be altered; that is, bone may be added to the periosteal or endosteal surfaces or it may be taken away from these surfaces. In *remodeling* all surfaces of the bone may be affected, including the internal body of the bone, by the formation of Haversian systems. In remodeling the bone involved is usually a small individual

packet called a basic nulticellular unit (BMU), and typically the amount of bone remaining after the remodeling cycle is little changed; new bone has more or less replaced old bone. This distinction will be seen to be important in chapter 11.

There is a type of secondary osteon that is different from the type described above; this is called the drifting osteon. It is found in younger animals, in general. After the cavity is formed by the cutting cone, it is not filled in at once; instead, one side continues to be eroded by osteoclasts, while the opposite side is filled in by osteoblasts. As a result the cylinder of the osteon drifts *sideways* through the tissue, and in so doing erodes and replaces a great deal of preexisting bony tissue (Robling and Stout 1999). Nothing is known about the mechanical consequences of the presence of such secondary osteons.

There is an outer sheath to the Haversian system, called the cement sheath (or line, because it is usually viewed in cross section). This is formed when the cutting cone stops its erosional activity and just before new lamellar bone is laid down on the raw surface so formed. The composition of the cement sheath is still controversial. Some, for instance, Frasca (1981), propose that it is more highly mineralized than surrounding bone; others, for instance, Schaffler et al. (1987), propose that it is less highly mineralized. There is agreement that there is very little collagen in it. Schaffler et al. produce evidence that it has more sulfur and less calcium and phosphorus than the neighboring bone lamellae, and is therefore probably less mineralized than them. It may be that the cement line is simply a very thin layer of osteoid that remains when the process of bone erosion is replaced by bone deposition (Martin and Burr 1989). Zhou et al. (1994) suggest that there are globular accretions (of unknown composition) on the ends of the degraded collagen fibrils that have partially eroded away, and that these accretions act as a bridge between the old degraded collagen fibrils, and the newly deposited ones. The cement line is also the site of a concentration of osteopontin, a bone protein that probably has some role in bone remodeling (Gerstenfield 1999; McKee and Nanci 1996; Terai et al. 1999).

It is a pity that this matter is not settled, because it is very important to know, for the purpose of understanding bone mechanics, whether cement sheaths are likely to be more or less compliant, and more or less strong, than the surrounding lamellae. Some canaliculi cross the cement sheath, so cells outside do have some metabolic connection with the blood vessel in the middle of the Haversian system. This is shown in figure 1.3 in which the blood vessels, osteocytes, and canaliculi are impregnated with resin and stand proud when the outer few microns of a specimen of bone are etched away. The density of canaliculi in bone is

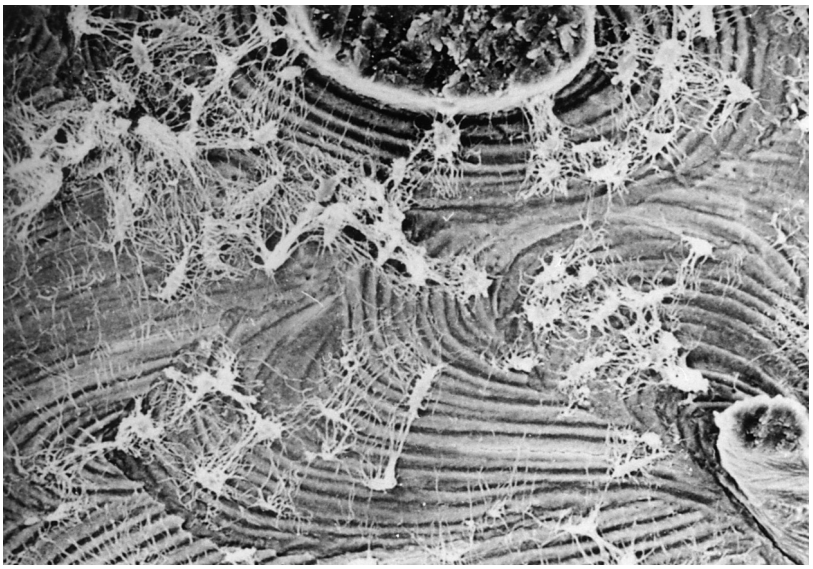

FIG. 1.3 Scanning electron micrograph of a resin-impregnated etched piece of human cortical bone. The resin stands proud of the bone. Two vascular channels can be seen (*top center, bottom right*). The lamellation of the bone is made obvious by the differential erosion produced by the etching acid. Osteocyte lacunae have radiating canaliculi, which can be seen passing over places where the lamellae obviously belong to two generations of osteogenesis, and which therefore have a cement sheath between them (*upper left*). However, the density of canaliculi crossing the sheath is generally less than the density nearby (bottom left, top right). The resorption and redeposition associated with the bottom right vascular channel starts at the bottom left of the picture. Width of image ~500 μm. (From a Christmas card kindly sent by Dr. Peter Atkinson.)

high, averaging about 5 canaliculi per hundred square microns in the plane normal to the predominant direction of the canaliculi (Marotti et al. 1995). The canaliculi account for a porosity of about 1.5% (Frost 1960). The cell biology of osteoclasis, the process of destruction of bone tissue, was reviewed by Zaidi et al. (1993).

A fourth characteristic type of mammalian bone is known as plexiform, or laminar, or fibrolamellar bone (Enlow and Brown 1956, 1957, 1958; Currey 1960; de Ricqlès 1977; Francillon-Vieillot et al. 1990; Stover et al. 1992). It is found particularly in large mammals, whose bones have to grow in diameter rather quickly. Lamellar bone cannot be laid down as fast as woven bone (as I said above, lamellar bone is laid down at a rate of less than 1 μm a day, while woven bone is laid down at more than 4 μm and, indeed, often much more, a day). If a bone has to grow in diameter faster than lamellar bone can be laid down, woven bone must be laid down instead. For reasons that will be discussed in

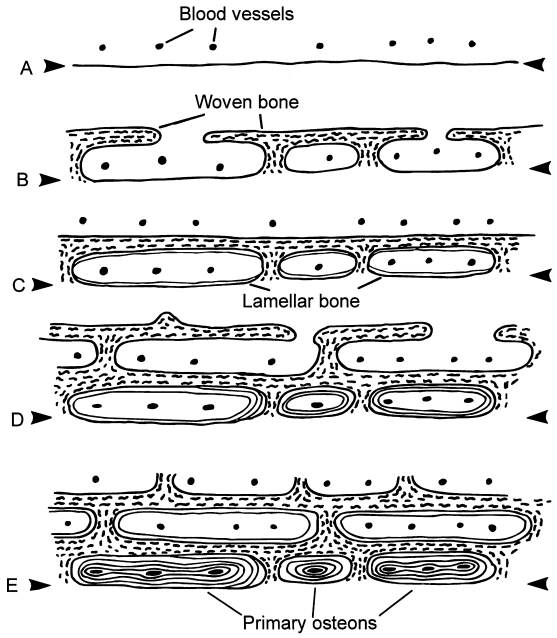

FIG. 1.4 The formation of fibrolamellar bone. These are cross sections of the outer surface of a rapidly growing bone. The arrowheads show the position of the original surface. Blood vessels are shown by black spots. (A) The original situation. (B) Woven bone, shown by squiggly lines, grows very quickly to form a scaffolding clear of the original surface. (Sometimes this "woven" bone may be much more like parallel-fibered bone.) (C) Lamellar bone, shown by fine lines, starts to fill in the cavities. (D) As more lamellar bone is laid down, so is another scaffolding of woven bone. (E) By the time the first row of cavities is filled in, forming primary osteons, the outer surface of the bone is far away.

chapter 3, woven bone is almost certainly inferior to lamellar bone in its mechanical properties. The undesirable mechanical results of having a bone made from woven bone are partially obviated by the production of fibrolamellar bone. Essentially, an insubstantial scaffolding of woven or parallel-fibered bone is laid down quickly to be filled in more leisurely with lamellar bone (fig. 1.4).

In bovine bone, each lamina is about 200 μm thick. In the middle a two-dimensional network of blood vessels is sandwiched between layers of lamellar bone. Beyond these layers, on each side, is a layer of parallel-fibered or woven bone, which is more heavily mineralized than the lamellar bone (fig. 1.5). In particular, there is a line in the middle, which is exceptionally heavily mineralized. This is the line at which growth stops for a short while before the next later is initiated. The way fibrolamellar bone is laid down means that there are, in effect, alternat-

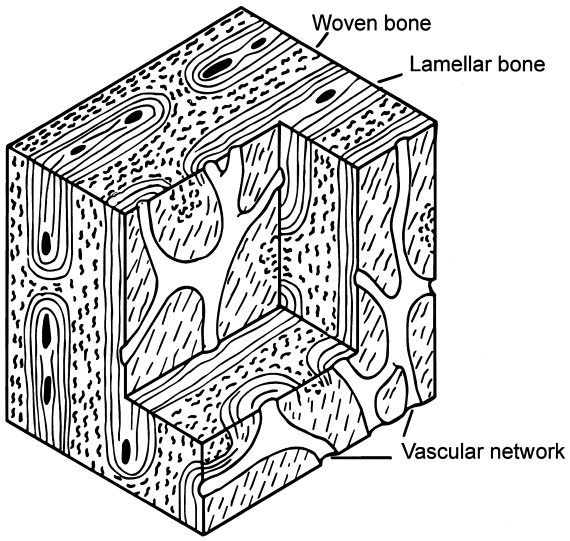

FIG. 1.5 Block diagram of fibrolamellar bone. Conventions concerning histological type as in Figure 1.4 Two-dimensional networks of blood channels, sheathed by lamellar bone, alternate with layers of woven bone.

ing layers of parallel-fibered or woven bone and lamellar bone tissue extending, quite often, for many millimeters, or even centimeters, in the radial direction. Fibrolamellar bone can be laid down vey rapidly. For instance Castanet et al. (2000) report that the femur of a young emu *Dromaius novaehollandiae* can be added up to 80 μm per day on the subperiosteal surface.

This description is of particularly neatly arranged laminar bone. Frequently the blood channels are more irregularly disposed or do not form a network, and the laminar arrangement gives way to one in which the blood vessels anastomose in three dimensions, and each is surrounded by more or less concentric layers of lamellar bone. This produces an appearance somewhat like that of Haversian systems, and the structures around the blood vessels are called *primary* osteons. However, there is a most important difference between primary osteons and secondary osteons, or Haversian systems: Haversian systems are *secondary*, that is, they replace bone that has existed previously. There are differences that enable one to distinguish Haversian systems from primary osteons histologically. In particular, secondary osteons are surrounded by a cement sheath, whereas primary osteons are not. Also, secondary osteons appear to drill through the preexisting bone, without regard to its structure, whereas the lamellae round primary osteons

merge smoothly with the surrounding bone. The distinction between the two types of osteon is not mere semantic hairsplitting, because differences between primary osteonal bone and Haversian bone correlate with differences in mechanical behavior, as we shall see. Haversian bone is weaker than primary bone. The distinction is frequently not made, and is a grave source of muddled thinking.

The kind of primary bone laid down depends on the rate of accretion. Castanet et al. (1996) related the changes in the histology of the bone of the mallard duck *Anas platyrhyncos* to the rate of accretion, which they determined by periodic labeling with a fluorescent dye. Different bones grow at different rates, and they were able to see the different kinds of histology being laid down at the same time, so what they found was not an aging or maturation effect. The humerus grew fastest and, initially, at seven weeks of age, had a rate of accretion of about 25 μm a day; the bone was completely fibrolamellar. As the rate of accretion diminished, the fibrolamellar bone gave way to anastomizing primary osteons. Finally, when the accretion rate was only about 1 μm a day, the blood vessels were sparse, all parallel to the long axis of the bone, and the bone consisted of circumferential lamellae. The phalanges, on the other hand, which had a much lower accretion rate right from the start, never showed any sign of fibrolamellar histology.

Stover et al. (1992) show that in bone that grows very fast in the radial direction, such as in the young foal, the outer sheet of woven bone may initially be connected to the rest of the bone by bony struts so sparsely, if at all, as to be effectively lying lie free in the periosteum. The outer sheet becomes connected to the rest of the bone only when mineralization is well underway. This process, which they call "saltatory primary osteonal bone formation," allows growth to be even more rapid than the process I described above. Indeed, Sue Stover tells me that, very occasionally, two layers of free-floating bone can be seen. However, it is obviously a somewhat risky process, because a blow to the periosteum would cause the relative positions of the outer sheet and the main bone to become deranged.

1.5 Primary and Secondary Bone

Primary bone is replaced by secondary bone in two ways: The bone can be eroded away at its surface, and then new bone can be laid down, or else Haversian systems can be formed. Enlow (1963, 1969) gives very clear descriptions of these processes. It is often quite difficult to tell when the former has happened, and the effects, if any, of such replacement on mechanical properties are uncertain. The adaptive reason (if it

is adaptive) for the formation of Haversian systems, which have a somewhat deleterious effect on mechanical properties, is obscure. The common explanation half-heartedly held by much of the bone community for a long time was that Haversian systems form when the bone mineral has, from time to time, to be released into the blood system for purposes of mineral homeostasis (Hancox 1972). Nowadays, mechanical explanations are more fashionable. There are various problems associated with such explanations that need not concern us yet, because we can accept Haversian remodeling as a fact and explore its mechanical consequences. I shall say much more, although rather inconclusively, about the function of Haversian remodeling in chapter 11.

The formation of Haversian systems tends to lead to the production of more Haversian systems. Each Haversian system is bounded by its cement sheath, and the passage of canaliculi across these cement sheaths is variable in amount (fig. 1.3), and is often rather sparse (Curtis et al. 1985). When it is poor, blood vessels will be separated from some of their catchment area, and osteocytes outside this area may find it difficult to obtain nutrients and are more likely to die (Currey 1960, 1964b). It is probably for this reason that the formation of a few Haversian systems in a region is often followed by the formation of many more in the immediate vicinity. Haversian systems often occur in clusters more often than would be expected by chance (Bell et al. 2000). Eventually, a region of bone may be completely occupied by Haversian systems and by luckless *interstitial lamellae*, little bits of bone that are separated by cement sheaths from all blood vessels, and so tend to be dead. However, death of bone cells by no means always leads to the formation of new bone to replace the old.

Human bone is like that of many primates and carnivores in that primary fibrolamellar bone is laid down initially, but this bone type is soon replaced by Haversian bone. However, this is not the case in many other mammalian groups. In most bovids (cattle) and cervids (deer), for example, the long bones keep their primary, fibrolamellar structure all through life, with only small regions, usually under the insertion of strong muscles, becoming Haversian. Many smaller mammals show no remodeling at all (Enlow and Brown 1958), the bone being fibrolamellar or, often, mainly composed of circumferential lamellae.

1.6 Compact and Cancellous Bone

At the next higher order of structure there is the mechanically important distinction between compact and cancellous bone. Compact bone is solid, with the only spaces in it being for osteocytes, canaliculi, blood

vessels, and erosion cavities. In cancellous bone there are large spaces. The difference between the two types of bone is visible to the naked eye. The material making up cancellous bone of adults is usually primary lamellar bone or fragments of Haversian bone. In young mammals it may be made of woven or parallel-fibered bone.

The structure of cancellous bone varies in three ways: in its fine-scale structure, in its large-scale structure, and in its porosity. At the lowest level, cancellous bone is usually made of lamellar, not woven bone. However, the lamellae usually do not usually run precisely parallel with the external surfaces of the trabecular struts and so they come out to the surface, rather like rocky strata coming to the surface of the earth, at odd angles. Singh (1978) has a convenient description of cancellous bone morphology at the next level. The simplest kind of cancellous bone consists of randomly oriented cylindrical struts, about 0.1 mm in diameter, each extending for about 1 mm before making a connection with one or more other struts (fig. 1.6), usually roughly at right angles. In a variation of this pattern the cylindrical struts are replaced by little plates. The amount of variation ranges from cancellous bone in which there is just the occasional plate among the struts to cancellous bone in which there is just the occasional strut among the plates. In other cancellous bone the plates may be considerably longer, up to several millimeters. When this happens there is a higher level of anisotropy: these longer plates are not randomly oriented but are preferentially aligned in one direction. The final form of such cancellous bone is shown in figure 1.6C, where there are parallel sheets of bone with fine struts joining them. Another type of cancellous bone consists almost wholly of sheets, forming long tubular cavities that interconnect by means of fenestrae in the walls. Gibson (1985) produced a somewhat idealized classification, which, though not grounded so firmly in the messy reality of life, is nevertheless convenient for mechanical modeling. This is discussed in Chapter 5. These different versions of cancellous bone as classified by Singh are found in characteristically different places. The type made of cylindrical struts, with no preferred orientation, is usually found deep in bones, well away from any loaded surface, while the more oriented types, made of many sheets, are found just underneath loaded surfaces, particularly where the pattern of stress is reasonably constant. If the trabeculae are more than about 300 μm thick, they often contain blood vessels, usually within secondary osteons (Lozupone and Favia 1990).

The porosity of cancellous bone is the proportion of the total volume that is not occupied by bone tissue. Usually it is filled with marrow, but in birds there may be gas. The porosity varies from being effectively complete, where there is only the occasional tentative strut sticking into the marrow cavity, down to about 50%. If the porosity is less than about 50%, then cancellous bone becomes difficult to distinguish from

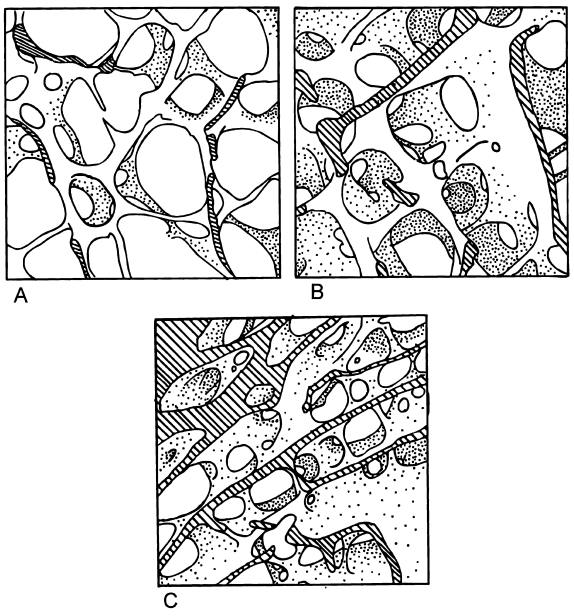

FIG. 1.6 Drawings of cancellous bone, seen by SEM. In each, the hatched parts are at the level of the top of the section. (A) Middle of the human sternum. Rather fine, nearly random network of mainly cylindrical struts. Width of picture 3.4 mm. (B) Human greater trochanter. Many of the elements are plates. Width of picture 3.4 mm. (C) Human femoral neck. The longitudinal plates are very obvious. There are many plates and struts lying orthogonal to them. Width of picture 8 mm (note smaller scale). ([A] Derived from Whitehouse [1975]; [B, C] derived from Whitehouse and Dyson [1974].)

compact bone with many holes in it. However, the change from compact to cancellous bone is usually clear and takes place over a small distance, and bone with a porosity of between 50 and 15% is uncommon. The mechanical reasons for this are discussed in chapter 5.

Bone grows by accretion on preexisting surfaces. Long bones have cancellous bone at their ends for reasons discussed in section 7.6. As long bones grow in length, cross sections that start off near the ends (and as the bone grows, move relatively closer to the middle of the length of the bone) usually undergo a *reduction* in diameter. This is because the ends (the epiphyses) are wider than the middle (the diaphysis) and although the diaphysis is slowly growing in diameter, the metaphysis (the part of bone just underneath the epiphysis) is usually reducing in diameter. The geometry of the situation is such that, quite often, compact bone has to be formed in a region where cancellous bone already exists. Here the old cancellous bone is not replaced; new bone is merely wrapped around the trabeculae, producing an extremely confused structure, with no obvious grain, called *compact coarse-*

cancellous bone. The effect of bone growth on bone histology and many other aspects of bone growth and structure (but not fine structure) are very clearly discussed by Enlow (1963, 1975).

1.7 A Summary of Mammalian Bone Structure

- *Tropocollagen molecules* (wrapped in a triple helix) lined up in files, and bonding side to side to form:
- *Microfibrils*, which aggregate to form:
- *Fibrils*, which are impregnated by and surrounded by the mineral hydroxyapatite or, somewhat more accurately, dahllite.

These fibrils appear in three different forms:

Woven bone	**Parallel-fibered bone**	**Lamellar bone**
Fibrils 0.1–3μm in diameter, arranged fairly randomly	Intermediate	Fibrils 2–3 μm in diameter, arranged in sheets (lamellae) 2–6 μm in thickness

Bone has bone cells, enclosed in *lacunae*:

Roughly isodiametric ~20 μm in diameter		Oblate spheroids, 5:1 ratio of major and minor axes. Major axes 20 μm

Canaliculi, about 0.2–0.3 μm in diameter, are channels containing cell processes that connect the cells with each other and with the nearest blood channel. Each osteocyte has about 60 canaliculi.

The bone is organized, at the next higher level, in four different ways:

Lamellar bone	**Woven bone**	**Fibrolamellar bone**	**Secondary osteons (Haversian systems)**
Often found in large lumps in reptiles. Found as circumferential lamellae in mammals and birds. *Primary and secondary*	Found in large lumps in young animals and in fracture callus. *Primary*	Alternating sheets of lamellar and woven bone/ parallel-fibered bone, with 2-dimensional nets of blood vessels. Ca. 200 μm between blood vessel nets. *Primary*	Cylinders of lamellar bone, solid except for a tube in the middle for blood vessels. Ca. 200 μm in diameter. *Secondary*

(continued on next page)

The bone is further organized into two different types of bone:

Compact bone	**Cancellous bone**
Solid, only porosity is for canaliculi, osteocyte lacunae, blood channels and erosion cavities.	Porosity easily visible to the naked eye. Rods and plates of bone, multiply connected, never forming closed cells.

1.8 Nonmammalian Bone

The bone I have discussed so far is mammalian bone. Rather little is known about the mechanical properties of nonmammalian bone, but I shall say something about its structure because of its interesting similarities and dissimilarities with mammalian bone. Many years ago Enlow and Brown wrote a useful summary of fossil and recent bone of all the vertebrates (1956, 1957, 1958). De Ricqlès produced a massive survey in ten parts of the histology of tetrapod bone, mainly that of reptiles. These papers are all cited in a comprehensive bibliography of 618 references, chiefly on bone histology, in de Ricqlès (1977). A (somewhat) shorter account is in Francillon-Vieillot et al. (1990).

The bone tissue of birds is like that of mammals, although at the naked eye level there are important differences in the proportions of wall thickness to overall diameter, which I shall discuss in section 7.3.2. However, the lamellae are usually less well developed than in mammalian bone, and the canaliculi have a much more wandering course (Rensberger and Watabe 2000). Some reptile bone is like mammalian bone; dinosaurs, in particular, often had well-developed fibrolamellar bone, Haversian systems, and a particularly rich blood supply (Currey 1962a). The bone of pterodactyls (pterosaurs) is more like that of birds (de Ricqlès et al. 2000). However, in many reptiles the bone is poorly vascularized and, indeed, is often avascular, although it does contain living bone cells. This poor vascularization is presumably possible because of the low metabolic rate of many reptiles. A characteristic of reptiles is the *lamellar–zonal* structure. This is bone principally made of parallel-fibered or true lamellar bone. It has poor vascularization and is particularly characterized by zones where growth comes to a halt then starts again. This pattern is characteristic of ectotherms, which often stop growing in the winter, and leads to lively debate among paleontologists trying to determine the physiology of extinct groups from their bone histology. The modern amphibia tend to have a rather simple, often avascular, bone structure. However, the earlier amphibia, such as the Embolomeri, which were quite large, show ill-developed lamellar–zonal or fibrolamellar bone, and also Haversian systems.

In the lower (less derived, in modern biology-speak) teleosts and in

lungfish there are bone cells, although there is a tendency for bone to be replaced by cartilage in these groups. However, the bone of most modern bony fish—the advanced teleosts—has no bone cells (Moss 1961b). This is a remarkable fact whose significance, physiological or mechanical, is obscure. The acellularity is brought about in different ways in different groups. In some the bone cells form in the ordinary way from osteoblasts, are incorporated into the bone, and then die, the lacunae they leave being filled up with mineral (Moss 1961a). In other groups the osteoblasts avoid being incorporated in the bone at all (Ekanayake and Hall 1988). Another remarkable feature of the bones of these fish is the way in which they hardly remodel. Fish bones appear to spread from centers of ossification in almost straight lines. (Cod skulls are cheap. Boil one for a while till all the flesh drops off, and you will see the striking difference between "ordinary" bones and the cod's skull bones. The bones themselves are extremely graceful.) In many species osteoclasts have never been observed, though they may be induced to develop by playing physiological tricks on the body chemistry (Glowacki et al. 1986), and sometimes rather peculiar osteoclasts are found naturally (Witten and Villwock 1997). If bones do not remodel, there must be considerable constraints on their functional adaptation.

Fish bone, despite being acellular, and in general not remodeling, can, as always in biology, produce splendid exceptions. The rostrum of the swordfish, which is made of true bone and probably has some hydrodynamic function, is acellular. Parts of it are intensely remodeled, showing dense Haversian tissue and sometimes little groups of secondary osteons surrounding a large blood vessel (Poplin et al. 1976). The lamellar structure characteristic of secondary osteons is not very well developed, and the whole tissue is completely devoid of osteocytes. This strange tissue's adaptations are a mystery.

Very little is known about the mechanical properties of fish bone tissue or of whole bones. One reason for this ignorance is interesting: it is very difficult to obtain specimens of teleost bone that are not pervaded with large, elongated cavities. These cavities, of course, make it difficult to prepare useful test specimens. The anatomy and physiology of teleost bone is a neglected subject. Since most vertebrate species are teleost fish, this is a shame.

Mineralized tissues in the vertebrates have been evolving for a long time; 450-million-year-old fossils of what are probably vertebrate tissues have been discovered in the late Cambrian (Young et al. 1996). The mineralized tissue of "lower" vertebrates, including extinct groups, is discussed by Ørvig (1967) and Francillon-Vieillot et al. (1990). The considerable range of histological structures seen in the nonmammalian vertebrates is a challenge, because so little is known of their mechanical properties. Undoubtedly, when fully investigated they will turn out to have instructive similarities to, and differences from, mammalian bone.