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PROSPECTS

Osteonal and Hemi-Osteonal Remodeling: The Spatial and Temporal Framework for Signal Traffic in Adult Human Bone

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Abstract The bone replacement process in the adult skeleton is known as remodeling. When bone is removed by osteoclasts, new bone is laid down by osteoblasts in the same place, because the load bearing requirement is unchanged. Bone is usually replaced because it is too old to carry out its function, which is mainly mechanical in cortical bone and mainly support for homeostasis and hematopoiesis in cancellous bone. Remodeling always begins on a quiescent bone surface, separated from the marrow by flat lining cells that are one of the two modes of terminal differentiation of osteoblasts. Lining cells are gatekeepers, able to be informed of the need for remodeling, and to either execute or mediate all four components of its activation-selection and preparation of the site, recruitment of mononuclear preosteoclasts, budding of new capillaries, and attraction of preosteoclasts to the chosen site where they fuse into multinucleated osteoclasts.

In cortical bone, osteonal remodeling is carried out by a complex and unique structure, the basic multicellular unit (BMU) that comprises a cutting cone of osteoclasts in front, a closing cone lined by osteoblasts following behind, and connective tissue, blood vessels and nerves filling the cavity. The BMU maintains its size, shape and internal organization for many months as it travels through bone in a controlled direction. Individual osteoclast nuclei are short-lived, turning over about 8% per d, replaced by new preosteoclasts that originated in the bone marrow and travel in the circulation to the site of resorption. Refilling of bone at each successive cross-sectional location is accomplished by a team of osteoblasts, probably originating from precursors within the local connective tissue, all assembled within a narrow window of time, at the right location, and in the right orientation to the surface. Each osteoblast team forms bone most rapidly at its onset and slows down progressively. Some of the osteoblasts are buried as osteocytes, some die, and the remainder gradually assume the shape of lining cells. Cancellous bone is more accessible to study than cortical bone, but is geometrically complex. Although remodeling conforms to the same sequence of surface activation, resorption and formation, its three-dimensional organization is difficult to visualize from two-dimensional histologic sections. But the average sizes of resorption sites, formation sites, and completed structural units increase progressively, as they do in cortical bone, indicating that the cancellous BMU travels across the surface digging a trench rather than a tunnel, but maintaining its size, shape and individual identity by the continuous recruitment of new cells, just as in cortical bone, a process that can be visualized as hemiosteonal remodeling. The conclusion that all remodeling is carried out by individual BMUs has important implications for bone biology, since many questions about how BMUs operate cannot be answered by studying either intact organisms or isolated cell systems. Many different steps in remodeling and many factors that influence each step have been identified, but very little is known about how the process is regulated in vivo to achieve its biologic purposes; most factors studied to date are likely permissive rather than regulatory in nature. Based on the proposed conceptual model of the BMU, much in vitro experimentation is relevant to the growth, modeling and repair of bone, but not to its remodeling in the adult skeleton. Further progress in the understanding of in vivo physiology will require the characterization of gene expression in individual cells to be related to the spatial and temporal organization of the BMU. This is likely to be possible only for osteonal remodeling in cortical bone in which, because of its geometric simplicity, individual BMUs can consistently be observed in two-dimensional, longitudinal sections.

Key words: BMU, cell recruitment, osteoclast, osteoblast, lining cells, bone micro-circulation, molecular histomorphometry

The bones undergo ceaseless change throughout life. In carrying out the functions of growth, maintenance, and fracture repair, osteoblasts build new bone and osteoclasts remove un-

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wanted bone. The importance of growth and fracture repair are obvious, but why should something that can survive for thousands of years after death need to be maintained during life? While growing, the bones change in shape and internal structure as well as in size, and bone is added and redistributed in response to continually changing mechanical demands. Bone is removed, not because there is anything wrong with it, but because it is needed in a different place; osteoclasts and osteoblasts are operating in the modeling mode [1,2]. When growth has ceased, the bones are fully adapted to support their mechanical functions; no further strengthening or realignment should be required, but *the turnover* of bone continues. Bone is removed, not because it is no longer needed in its present location, but because for some reason it has to be replaced; osteoclasts and osteoblasts are operating in the remodeling mode [1–3].

The reason for replacement is different in cortical and in cancellous bone. Like other load-bearing structural materials, cortical bone is subject to fatigue damage, which can be forestalled by periodic replacement [1,2]. An acceptable level of bone aging could be maintained by non-directed stochastic remodeling, but already damaged bone is removed by directed remodeling targeted to a specific location [4]. Cortical remodeling also provides a means for meeting a temporary demand for more calcium during pregnancy, lactation, adolescence, and antler growth in deer [5]. Cancellous bone is also load-bearing, but is more concerned with short-term calcium homeostasis. As bone ages, its water is displaced by enlargement of the crystals, so that the mineral becomes progressively less accessible to exchange with the extracellular fluid [6]. Cancellous bone also provides essential support for hematopoietic tissue [7]; perhaps the support is not merely mechanical but also chemical, needing the continued outward diffusion or periodic release of molecules buried in the matrix. It is not known whether cancellous bone remodeling is purely stochastic and nondirected, or whether, as in cortical bone, it must from time to time be directed to a specific region, but it is likely that both types of replacement occur throughout the skeleton [8].

BONE REMODELING: THE ESSENTIAL ROLE OF BONE LINING CELLS

The purpose of bone remodeling as a replacement mechanism dictates its manner of operation [1,3]. The process invariably begins on an existing bone surface—the necessary cells (osteoclast and osteoblast precursors) arise from marrow tissue adjacent to bone, not from within bone itself [9]. A site where the bone surface is quiescent with respect to remodeling is selected for initiating the process; whether remodeling is directed or nondirected, each episode begins at a particular place at a particular time. For intracortical remodeling, the process originates on the wall of an Haversian or Volkmann canal [10], and the necessary cells travel to the selected site via the circulation. If the site of origin is adjacent to hematopoietic bone marrow, the necessary cells could migrate to the bone surface directly without entering the circulation, but neo-angiogenesis is probably an important component of all bone remodeling [1,11], providing a common basis for cell transport. At the selected site, bone resorption occurs first; before something can be replaced, it must be taken away, so that osteoclasts are the first cells to arrive. Formation of bone occurs later at the same site, where it is still needed, since the load-bearing requirement is unchanged. Osteoblasts follow osteoclasts after a brief interval, normally appearing only at sites where bone resorption has recently ceased. In a young adult, all the bone removed is replaced, and at the completion of the cycle the bone surface is restored exactly to its initial location [1,3].

The quiescent or resting regions of bone surfaces, where neither resorption nor formation is currently in progress, occupy about 75% of the total surface adjacent to bone marrow and about 95% of the intracortical surface in the long bones of the extremities. The quiescent surface is covered by a single layer of thin, flat, extended cells that separate the bone from the adjacent soft tissues [12]. These cells are commonly, but confusingly, referred to as osteoblasts. They are members of the osteoblast family, representing one of the two states of terminal differentiation of osteoblasts, the other being osteocytes buried within the bone. But as in other branches of biology, cells of the same lineage that differ in morphology and function deserve their own name; the cells that cover the quiescent regions of bone surface should be referred to as lining cells, since they no longer carry out the principal function of osteoblasts, which is to make bone matrix [13]. Between the lining cells and the bone is a thin layer of permanently unmineralized collagen-rich matrix—the endosteal mem-

brane [14]. Although its existence in adult bone has been questioned, the endosteal membrane has been convincingly demonstrated by transmission electron microscopy in dog femur and radius [12], and by scanning electron microscopy in human rib [15].

The importance of lining cells in the present context is that they are the gatekeepers for the initiation of bone remodeling [2,3,13,14,16]. Frost originally coined the term "activation" for the first step in starting a new remodeling cycle, which he regarded as the delivery of a mitogenic stimulus to mesenchymal cells, the term then in use for the putative pluripotential stem cells that were believed to give rise, inter alia, to osteoclasts and osteoblasts [17]. It is now frequently stated that the first step in remodeling is "activation" of osteoclasts, implying the conversion of osteoclasts from an inactive to an active state. This is clearly incorrect, since when a region of quiescent bone surface is already committed to undergo remodeling, there are no osteoclasts present at that location. The notion that osteoclasts are in need of activation arose from experiments in young, rapidly growing rats [18], but in adult human bone, in a remodeling rather than in a modeling situation, there is no evidence that inactive osteoclasts exist [3]. When new osteoclasts appear at the right place and time, they begin their work immediately, and when their work is finished, they disappear.

Remodeling activation is best defined as the conversion of a region of bone surface from quiescence to activity; it is important that this usage of the term in remodeling theory not be confused with activation of cells or of molecules. The alternative term "initiation" has been recommended to minimize confusion, but conveys less well the sense of a critically important event, which is analogous to the switch from G_0 to G_1 of the cell cycle in discontinuously replicating tissues [3]. Whatever term is used, the process includes four components [3,16]. The first is selection and preparation of the site; for directed remodeling this will presumably be the site closest to the target. The second is recruitment of mononuclear preosteoclasts, presumably requiring the completion of differentiation by precursor cells; depletion of the precursor cell pool is the most likely signal for its replenishment, by division of the appropriate stem cell or colony forming cell, a process that need not be directly linked to activation. The third is budding of new capillaries; this is definitely required for cortical remodeling but might not be essential for cancellous remodeling. The fourth is attraction of preosteoclasts to the chosen site, where they fuse into osteoclasts [19].

The only cells that are strategically placed to coordinate these different components are the lining cells, the members of the osteoblast family that carry out in vivo the various functions of osteoblasts in facilitating bone resorption that have been demonstrated in vitro [16]. Lining cells can both receive and deliver signals, from and to many other cells, including those in the adjacent soft tissue, those elsewhere on the surface of bone, more distant cells via the circulation, and, because of their contact with osteocytes via the canaliculae, cells within the bone itself. Lining cells secrete collagenase to digest the endosteal membrane and so expose the bone mineral [20], and change their shape to allow access of osteoclast precursors to the mineralized bone [14], to which they are attracted by various mechanisms. These include chemotactic signals such as calcium and osteocalcin [3] and immunologic recognition, possibly involving locally expressed antigens and circulating T lymphocytes [9]. Because of their origin and location, lining cells are able both to be informed of the need for remodeling, and to either execute or mediate all four components of its activation [2,3,16].

Osteonal Remodeling

Cortical bone is currently an unfashionable subject of study. Measurements on appendicular cortical bone laid the foundation for the understanding of age-related bone loss as a biologic phenomenon, but are now rarely performed in clinical practice. Much of what is known about bone remodeling as an integrated process derives from studies on cortical bone carried out more than 30 years ago [1,21,22], but most investigators in the bone field today have ignored, forgotten, or never learned about this fundamental work. A fully developed cortical remodeling unit (Fig. 1), or Basic Multicellular Unit (BMU) in Frost's terminology [1], is an elongated cylindrical structure, about 2 mm long and 0.2 mm wide, that burrows through bone, in a direction generally aligned with the long axis of the bone, at a characteristic rate (20–40 μm/day) for a variable distance (2–6 mm) [10]. During its lifespan of 6–12 months, the spatial and temporal relationships between its components are preserved, and it is continuously steered in

an appropriate direction. The maintenance of this unique entity requires not only the continued sequential recruitment of new osteoclasts and osteoblasts, in the right numbers, and at precisely defined but ever-changing locations, but the growth of new blood vessels, nerves, and connective tissue at rates commensurate with the progression of the entire structure. The end result of each new BMU is one new Haversian system or osteon. At the boundary between the cylinder of new bone and the surrounding old bone is a thin layer of cement substance, visible in cross-sections as the cement line.

At the forward end of the BMU is the cutting cone, about 0.2 mm long, where about nine osteoclasts each containing about nine nuclei [23] are resorbing the bone in front of them. Behind the cutting cone is a transitional or reversal zone about 0.2 mm long, lined with spindle-shaped cells, where cement substance is deposited on the wall of the cavity. Further behind is the closing cone about 1.6 mm long, lined with about 2,000 osteoblasts forming bone within the cavity [24]. In the center is a capillary loop and supporting connective tissue. Because the entire structure is advancing, increasing *distance* from the apex of the cutting cone corresponds at each location to increasing *time* since the apex was at that location (Figs. 1,2). The succession of events at a single cross-section, from the beginning of resorption proceeding centrifugally to the end of formation proceeding centripetally, represents one cycle of remodeling. For convenience in measuring their birthrate by tetracycline-based kinetics, the cycles are usually defined in terms of an arbitrary length of bone [10,21], but it makes more biological sense to think of the cycles in terms of cell recruitment [22]. Because the osteoclasts move forward but the osteoblasts remain in the same cross-sectional location, each successive ring of new osteoblasts lining the circumference of the cavity can be regarded as belonging to one remodeling cycle.

The 80 or so osteoclast cell units in the cutting cone constitute a team, in the sense that they are working together to accomplish a common task. Their turnover, measured by tritiated thymidine labeling, is about 8%, or about seven nuclei, per day, corresponding to a mean transit time of about 12.5 days [23]. An individual osteoclast could maintain its identity for the entire

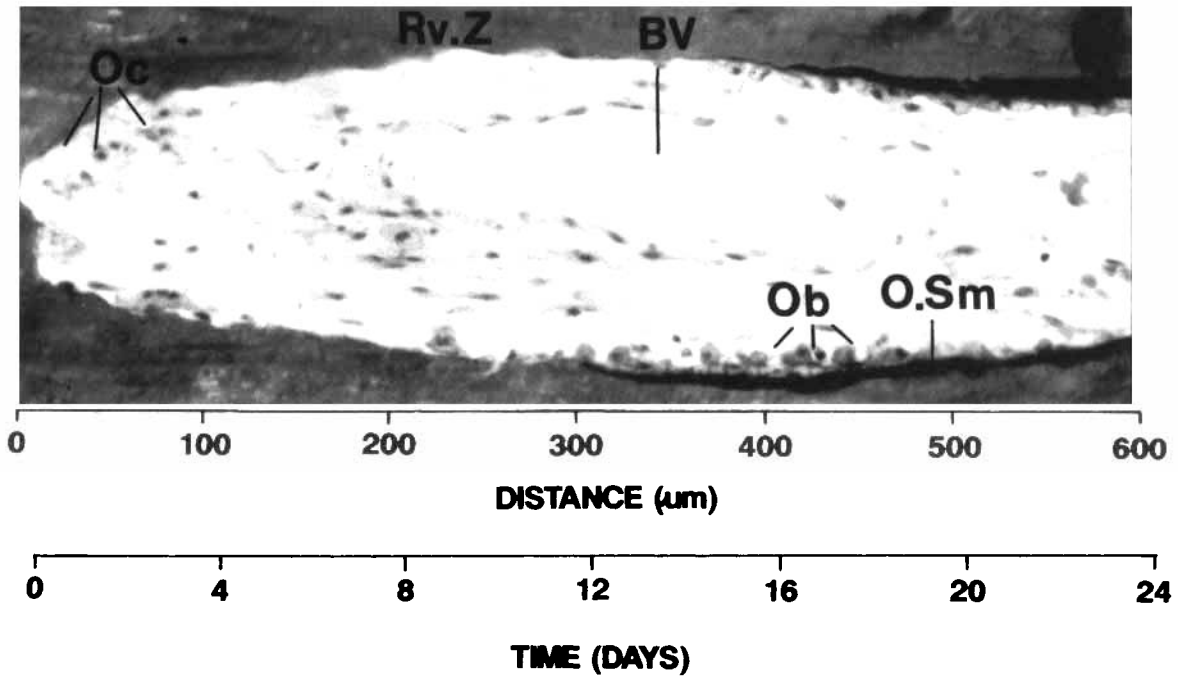

Fig. 1. Cortical BMU in normal human iliac bone. The structure is traveling from right to left; the distance and time scales (measured from the apex) are based on an indirectly estimated rate of advance of 25 μm/day (10). In front is the cutting cone of osteoclasts (Oc), followed by the closing cone behind lined by osteoblasts (Ob) laying down bone matrix as an osteoid seam (O.Sm), which extends the full length of the closing cone (not shown). In between is the reversal or transitional zone (Rv.Z), and in the center is a thin-walled blood vessel (BV), either a sinusoid or a large capillary.

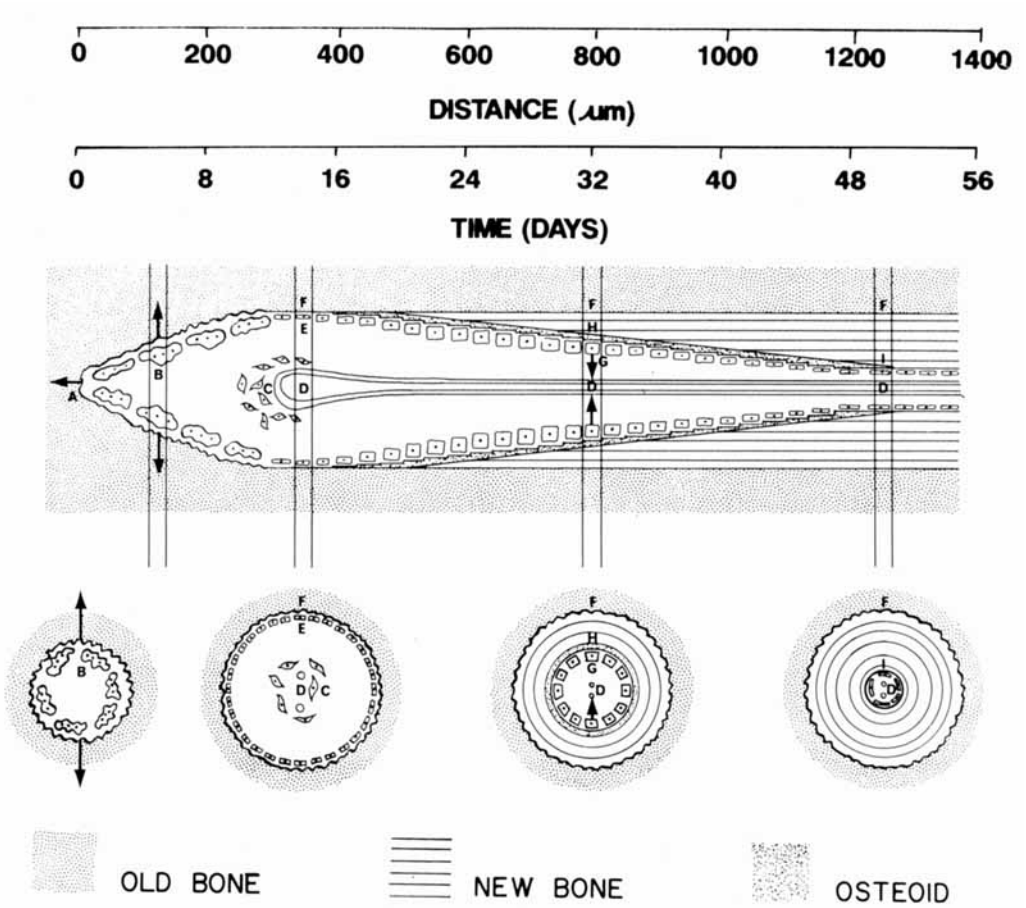

Fig. 2. Diagram of cortical BMU. Correspondence between longitudinal section above and transverse sections below, showing successive stages in the remodeling cycle. The distance and time scales (measured from the apex) are based on advance at 25 μm/day, as in Figure 1. A, apex of cutting cone moving from right to left; B, multinucleated osteoclasts enlarging the resorption space; they are depicted as eroding centrifugally, although forward erosion is just as likely (10); C, location of dividing precursors of preosteoclasts and preosteoblasts; D, capillary loop; E, mononuclear cells lining the reversal zone (location of dividing preosteoblasts); F, cement line separating new bone from old; G, osteoblasts advancing centripetally; H, osteoid seam separating osteoblasts from recently formed bone; I, lining cells at periphery of canal of completed Haversian system. For convenience of depicting the entire structure, the slope of the closing cone is about twice as steep as normal. Reproduced from Parfitt [21] in modified form, with permission of the publisher.

duration of the BMU, but would change all its constituent nuclei many times over. Nuclei leave by apoptotic death [25] and are replaced by the random fusion of new pre-osteoclasts. Their precursors, presumably originating in the bone marrow and leaving the osteonal capillary by diapedesis, divide locally in the connective tissue within the cutting cone [23]. It takes about 3.5 days for the daughter cells to differentiate, migrate, and join the osteoclast team, so that the average lifespan after the last division is about 16 days. A comparably short life span and consequent need for constant replenishment is found also in cells of the skin, intestinal mucosa, and most of the formed elements of the blood. In such tissues, health and disease depend more on maintaining, or failing to maintain, an adequate supply of new cells, than on manipulations or derangements of their function [26].

For osteoclasts the team includes cell units of different ages, but for osteoblasts the team comprises cells all of the same age. New osteoblasts arrive at the cement surface only in a narrow collar between the transitional zone and the closing cone [24]. As the cutting cone advances, a succession of new osteoblast teams are recruited, at the frequency required to ensure that the closing cone remains the same distance behind the cutting cone. The osteoblasts are derived from spindle-shaped precursors lining the circumference of the transitional zone that divide at least twice before becoming osteoblasts 1–2 days later [24]. The spindle cells are derived from larger dividing cells, more ovoid in shape,

located closer to the capillary. These could be stem cells that reside permanently in the connective tissue of the canals, or, like osteoclast precursors, they could originate in the bone marrow and cross the capillary wall. The absence of labeled osteoblasts along the length of the closing cone indicates that, in order to make enough bone to narrow the cavity to the diameter of a mature haversian canal (about 40 μm), all the osteoblasts that are needed for each team must be present before bone formation begins. New osteoblasts must assemble in the right place within a brief window of time; if they are late, they can join the next team, but the previous team will be permanently short-handed [27].

Because of the relationship between distance and time (Figs. 1 and 2), how osteoblasts change during their life span can be inferred from examination of cross-sections, using distance from the cement line, in conjunction with tetracycline labeling, to indicate time since the onset of bone formation at that location [21,27,28]. The osteoblasts, initially columnar or cuboidal in shape, begin rapidly to make bone matrix, which is called osteoid until it begins to mineralize some days later. The osteoblasts become progressively flatter and thinner, and cover a progressively larger area until finally they complete their morphologic transformation to the lining cells that cover the wall of the mature haversian canal. At the same time, the rate of matrix apposition declines, mainly because the secretory territory for which each osteoblast is responsible gets larger, but partly because the vigor of the cells diminishes as they get older [27]. The osteoid seam gets thinner and is eventually replaced by the endosteal membrane. The life span of an osteoblast while it is making bone varies from a few days to about 3 months, depending on the timing of incorporation into the new bone as an osteocyte. The life span of a lining cell or an osteocyte in cortical bone varies from a few years to several decades. But many of the osteoblasts initially present cannot be accounted for and presumably die [27]. The total loss of cells from the surface is greater than the fall in circumference, accounting for the shape changes described earlier. It is not known whether selection among the three possible fates of an osteoblast is determined during differentiation, or occurs at random.

Hemi-Osteonal Remodeling

The current preoccupation with cancellous bone has many roots. Changes with age are more rapid than in cortical bone and are much easier to measure than in the past. The ilium has several advantages over the rib as a biopsy site—the procedure is easier and safer, and because of proximity to hematopoietic tissue, turnover is higher and deviations from normal occur sooner and are of larger magnitude. Most in vitro systems, whether of organ or cell culture, resemble more closely the situation in cancellous bone than in cortical bone of the adult human skeleton. But cortical bone has a unique advantage for the study of remodeling—the investigator can control the orientation of a histologic section to the structure of interest. The geometry of cancellous bone is complex and section orientation is unpredictable. The average remodeling history of a representative point on a surface can be reconstructed [27,28], but the discrete, quantal nature of remodeling is obscured. The individual BMU, such an obvious and tangible phenomenon in cortical bone, becomes a difficult abstraction that can no longer be related clearly to the histologic appearances. Scientists, whether basic or clinical, who are acquainted only with cancellous bone can have no conception of how the events of remodeling are related to each other in three-dimensional reality.

It is now widely accepted that cancellous bone remodeling operates in accordance with the sequence activation—resorption—formation, but the acceptance is often based more on conformity to fashion than on personal conviction. Few investigators understand all the implications of quantal remodeling theory concerning the pathogenesis, diagnostic evaluation, and treatment of metabolic bone disease, and even fewer can cite the evidence on which the theory was founded. The most compelling evidence, presented earlier, derives from the study of cortical bone, but the differences between cortical and cancellous bone are mainly geometric rather than biological. There is no physical necessity for resorption to precede formation, as there is in cortical bone, but cancellous bone also needs a replacement mechanism. When the configuration of cement lines was extensively examined, the great majority were irregular and scalloped, indicating reversal from resorption to forma-

tion, and very few were smooth, indicating the initiation or resumption of formation without prior resorption [29]. It can be argued that such evidence is subjective and open to influence by prior expectation, but inspection of the detailed topography of cancellous bone leads to the same conclusion [14,30]. Although direct transformation of a quiescent to a forming surface without intervening resorption is possible under some circumstances [1,27], this cannot be what ordinarily happens.

The usual model of cancellous bone remodeling depicts the downward erosion and upward refilling of a cavity, moving in directions perpendicular to the bone surface, and culminating in a new bone structural unit, corresponding to an osteon in cortical bone [3,27,31] (Fig. 3). Such a simple model is convenient for illustrating the cyclical nature of the process and for analyzing the cellular basis of bone loss, but engenders the false belief that osteoclasts and osteoblasts are never present at the same time at the same remodeling site. A central feature of the BMU has been lost, and with it the possibility of thinking clearly about the pathways of intercellular communication. The notion that a BMU travels across the surface of cancellous bone, digging a trench rather than a tunnel, was discussed inconclusively at the First Histomorphometry Workshop in 1973 [32] and has been revisited on several occasions since [10]. Frost gave the first explicit endorsement of the notion, suggesting that the organization of a cancellous BMU could be visualized as the lower half of a cortical BMU [1]. Eriksen first depicted a plausible three-dimensional structure for a cancellous BMU (Fig. 4) (28); he did not take the crucial extra step of adding a distance scale to the corresponding time scale, but clearly had in mind a direct comparison with a cortical BMU [33].

Conclusive evidence for movement across the surface is provided by the dimensions of structures at different stages of remodeling. Accord-

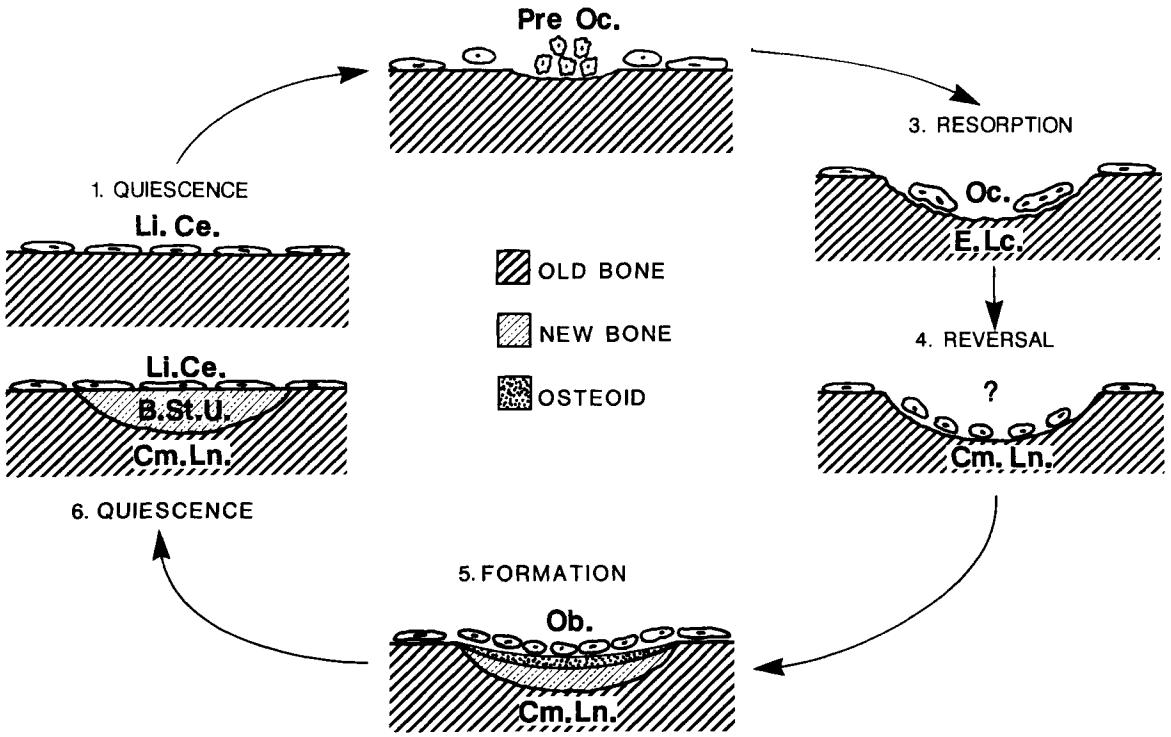

Fig. 3. Diagrammatic representation of the remodeling cycle in cancellous bone. Successive stages of quiescence, activation, resorption, reversal, formation, and back to quiescence at a single cross-sectional location are depicted. Li.Ce = lining cell; Pre Oc. = preosteoclast; Oc. = osteoclast; E.Lc. = eroded lacuna; Cm.Ln. = cement line; Ob. = osteoblast; B.St.U. = bone structural unit. Refilling is assumed to be complete, and bone marrow lying above the lining cells is omitted for clarity. Reproduced from Parfitt [31] with permission of the publisher.

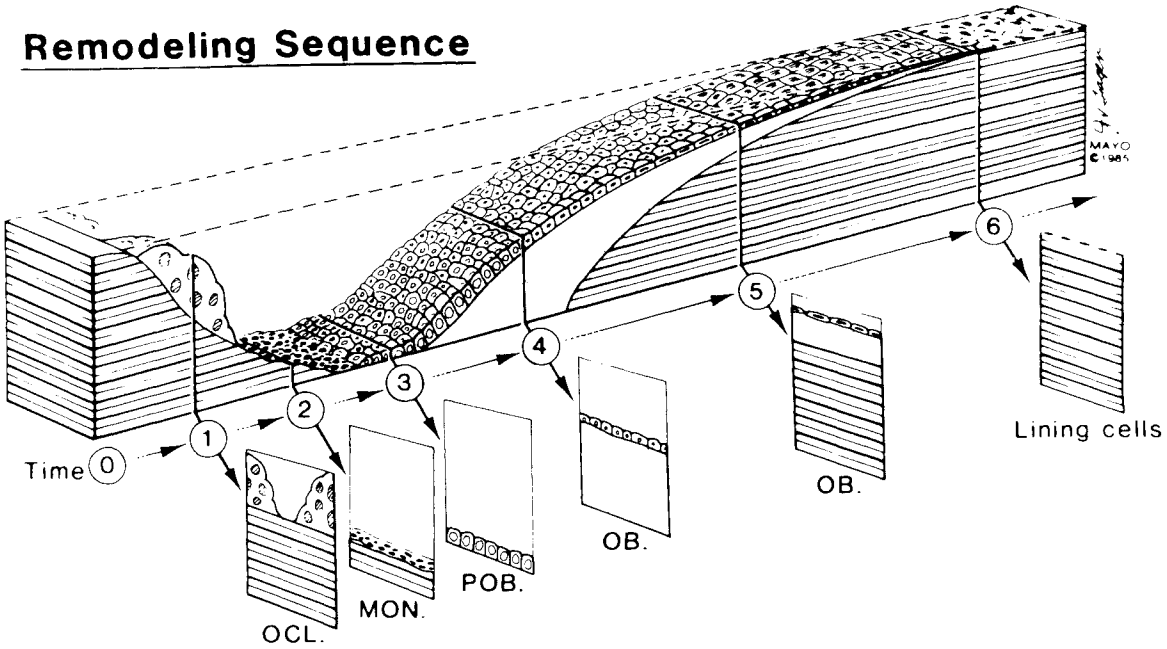

Fig. 4. Diagram of likely three-dimensional organization of a cancellous BMU. The numbers refer to the temporal succession of events at a single location, but with the addition of a distance scale they can depict events occurring simultaneously at different locations. O, old bone with quiescent surface. 1. Early bone resorption with osteoclasts (OCL). 2. Late bone resorption with mononuclear cells (MON). 3. Reversal zone with preosteoblasts (POB). 4. Early matrix formation by osteoblasts (OB) before onset of mineralization. 5. Late bone formation by osteoblasts after onset of mineralization. 6. Completed new hemi-osteon covered by lining cells. Reproduced from Eriksen [28] with permission of the publisher.

ing to the simple up and down model, the distances across a resorption cavity, an osteoid seam, and a completed structural unit should all be about the same, but their average profile lengths in histologic sections increase successively, just as they do in cortical bone (Table I) [31]. A cancellous bone structural unit reconstructed in three dimensions from serial sections is of irregular but elongated shape and up to about 2–3 mm in greatest dimension [34]; it was termed a trabecular osteon, but hemi-osteon would be more descriptive. Such a structure could be made only by a remodeling process that moved across the surface. This conclusion does not by itself establish how osteoclasts and osteoblasts are related to each other within a cancellous BMU, but Schenk et al. [30] has observed structures that correspond exactly with those postulated by Frost (Fig. 5). Such an appearance is rare for several reasons; randomly oriented sections are more likely to cut across than along an elongated structure, the complex curvature of the surface requires precise orientation of the section plane, and the BMU will often be constrained by the local topography to change its direction of advance.

The process of remodeling is fundamentally the same in cortical and in cancellous bone. In both, the BMU maintains its size, shape, and individual identity as it moves through or across the bone, requiring the continuous successive recruitment of new cells. During its longitudinal advance it creates and leaves behind a succession of transversely operating remodeling cycles, each new one slightly out of step with the one before. There is the same relationship between distance and time, so that events occurring at

TABLE I. Serial Dimensions During Remodeling*

Structure	Cancellous bone[a]	Cortical bone[b]
Resorption cavity	230	400
Osteoid seam	550	1,600
Complete BSU[c]	860[d]	3,000

*Data are presented in μm. Values in cancellous bone are consistent with sections through elongated structures that are about 80% as large as in cortical bone.

[a]Mean perimeter lengths in randomly oriented sections; original sources in Parfitt [31].

[b]Approximate structure lengths in longitudinally oriented sections; original sources in Parfitt [10].

[c]Bone structural unit; hemi-osteon in cancellous bone, osteon in cortical bone.

[d]The value approximately twice as large given in Parfitt [31] was based on a misreading of the original source.

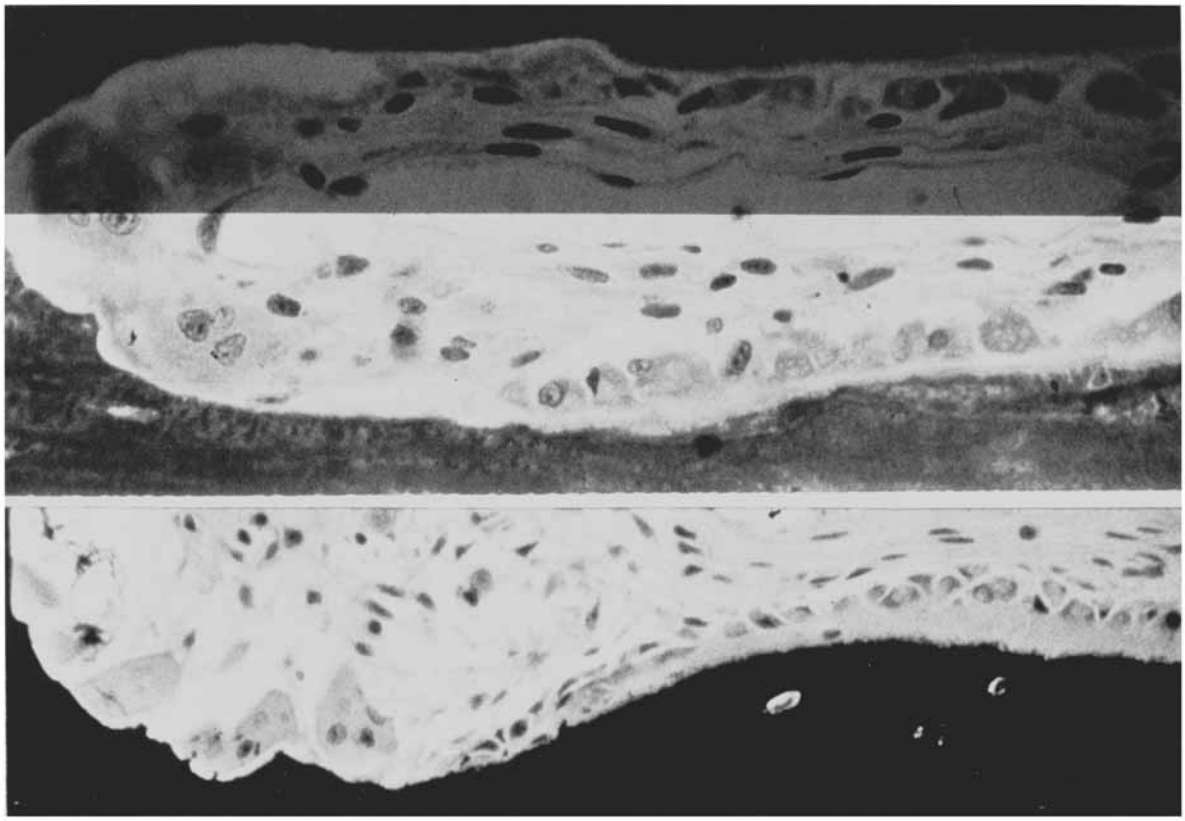

Fig. 5. Osteonal and hemi-osteonal remodeling. Upper panel shows a representative cortical BMU in the dog, with the upper half shaded. Lower panel shows a cancellous BMU in a patient with secondary hyperparathyroidism. Although it is possible that the appearance is specific to the particular disease state in which it was observed, reasons are given in the text for believing that the structure is characteristic of cancellous bone remodeling in general, but is likely to be observed only when bone turnover is very high, because the necessary circumstances, described in the text, will otherwise rarely occur. Called to author's attention by Dr. David Baylink. Original provided by Dr. Robert Schenk, and reproduced with permission.

the same time but at different locations correspond with events occurring at the same location but at different times; these events occur in the same sequence as depicted in the up and down model (Fig. 3). Much less is known about the kinetics of cell recruitment and turnover in cancellous than in cortical bone, but there is no reason to doubt that osteoclast nuclei have a short life span and are continually replaced, and that successive teams of new osteoblasts assemble, at the junction between the reversal zone and the osteoid seam, within a brief window of time. The osteoblasts undergo the same sequential changes in morphology and function, and the same three fates, as in cortical bone [27]. The fates are not in the same proportion, because of the difference in geometry, which requires survival of more cells in cancellous than in cortical bone, and more lining cells relative to the number of osteocytes.

The differences between osteonal and hemi-osteonal remodeling are those imposed by geometry. In cancellous bone, the full extent of the BMU is probably exposed to the marrow; the lining cells could persist as a canopy over the BMU, but it is more likely that they are destroyed. BMUs are much closer together, in both space and time, adjacent to hematopoietic marrow, with its abundance and diversity of cells and high blood flow, than adjacent to fatty marrow [35]. In the former case, the cancellous BMU could make use of existing blood vessels, but in the latter case a new capillary loop is required behind the advancing osteoclasts; such an arrangement would provide for the same kind of vascular communication within all types of BMU. Whether the BMU includes new nerves and connective tissue, as well as new blood vessels, is unknown. In osteonal, unlike hemi-osteonal, remodeling there is loss of contact with the surface of origin, which as previously noted was the wall of an intracortical canal. Although a cortical BMU can proceed along an existing haversian canal, more often a new os-

teon is made with a canal more or less parallel to the old one, at least in the long bones. In cortical bone of the axial skeleton, as exemplified by the ilium, there appears to be no preferred orientation for osteonal remodeling [34].

QUANTAL REMODELING THEORY AND BONE BIOLOGY

Bone remodeling is carried out by individual BMUs, which are anatomically discrete and functionally complex; many questions arise about how they operate. Some questions concern the external controls that regulate their location, birth rate, life span, number, speed of advance, and direction and distance of travel. Other questions concern the internal controls that regulate their size, shape, maintenance, coordination, and efficiency. The distinction between the external and internal control mechanisms is not absolute, since they must overlap and interact in supporting the mechanical and metabolic functions of the bones. None of the questions about BMUs can be answered by studying either intact organisms or isolated cell systems. It was to call attention to this fact that Frost coined the term "skeletal intermediary organization" [1], but his views have had regrettably little impact on the conduct of bone research. Many investigators continue to believe that everything of biological importance about bone remodeling can be captured by just two numbers. This belief is equally naive, whether the numbers refer to biochemical indices of whole body bone resorption and formation, as used in clinical investigation, or to treated/control ratios for measurements such as radiocalcium release or labeled proline incorporation, as used in basic laboratory research.

Writers of introductions and editorials in the bone field commonly indulge in a great deal of mutual back slapping. So much progress has been made, they imply, that final answers must be just around the corner. The truth, rarely admitted, is that we still have only fragmentary understanding, at the cellular and molecular levels, of the most important features of bone remodeling in vivo. We do indeed know much more now than a decade ago about the steps that lead from division of the relevant stem cells to the disappearance and reappearance of a small moiety of bone [9,36]. But we know very little about how these pathways are regulated to achieve the biological purposes of remodeling [2], and even less about the mechanisms whereby the recruitment and function of individual cell units are coordinated within a BMU. A multitude of factors has been discovered that can affect, positively or negatively, one or more of the steps involved in bone remodeling [9,36], but not one of these factors has been demonstrated to participate in its in vivo regulation. For example, calcitonin is a potent inhibitor, and IL-1 is a potent stimulator, of osteoclastic bone resorption, but there is no evidence that either of these effects has a physiologic function, nor that changes in calcitonin secretion or IL-1 production are components of any mechanism of physiologic control. Pathologic increases or decreases in the supply of these and many other factors may contribute to bone disease, but the effects of variations within the usual range are unknown.

Progress in the understanding of remodeling has been slow for several reasons. Despite the abundance of in vivo evidence that manipulation of cell recruitment is much more important than manipulation of individual cell function, the latter has attracted disproportionate attention. But none of the innumerable short-term changes in cell activity that can be experimentally induced have been shown to affect the quantity of remodeling work that is ultimately carried out. Even those few investigators who have recognized the overriding importance of cell recruitment have often used inferior methods, manifesting ". . . the stubborn misconception that the uptake of tritiated thymidine necessarily measures DNA synthesis, rather than some of the several alternative possibilities, . . ." [37]. Another reason is the seductive technical simplicity of molecular biology. It is a straightforward matter to demonstrate that one cell type expresses the gene for a particular molecule, and that another cell type expresses the gene for a receptor to which that molecule can bind. But this establishes only that a particular signal pathway is *possible*, not that the pathway actually exists in vivo, still less that the pathway participates in physiologic regulation. Many of the factors that can produce in vitro effects on bone cells most likely have a permissive rather than a regulatory role, contributing to a biochemical ambience that enables and supports the operation of control mechanisms that remain unidentified.

Most in vitro work is relevant, less to bone remodeling than to bone growth, modeling, and repair. Understanding these processes is vital,

because of their intrinsic importance, but also because it will help to define the range of possibilities for bone cell behavior with which any viable theories of remodeling must be consistent, and will provide new insights for the interpretation of ex vivo observations. For example, consider the sequential changes in gene expression that underly the proliferation and differentiation of osteoblasts in culture [38]. The later stages in the sequence illuminate the morphologic and functional changes during the life span of adult human osteoblasts that have been deduced from tetracycline-based histomorphometry [27]. The earlier stages, however, are, at first sight, inconsistent. In vitro, collagen synthesis and cell proliferation occur concurrently [38], but in vivo, collagen synthesis does not begin until cell proliferation is completed [27]. But this difference can be reconciled in the following manner. During the formation of woven bone, the two stages could be telescoped in the interest of speed but at the expense of precision of molecular alignment and orientation, whereas during the formation of lamellar bone, the two stages must be separated in the interest of precision but at the expense of speed. Nevertheless, even a complete understanding of bone growth, modeling, and repair will not by itself answer any of the questions posed earlier about the operation of BMUs.

Osteoporosis is of great interest to the public, to politicians, and to dispensers of research funds. Applicants for research support in the bone field almost always include some reference to osteoporosis in the "significance" section of their proposal. Fracture risk is influenced by peak adult bone mass, to which an understanding of bone growth is highly relevant. But age-related bone loss is a disorder of bone remodeling, and a full understanding of its pathogenesis is possible only in terms of normal BMU physiology. How can the methods of cell and molecular biology be deployed to study a biologic phenomenon that is manifested only in the intact organism? A useful beginning would be for investigators to have a clearer mental picture of what is actually going on. For reasons given earlier, cancellous bone provides a more appropriate framework than cortical bone for in vitro biologists to think about remodeling [39,40], and a model of a hemi-osteonal BMU is depicted in Figure 6. Because of the spatial and temporal relationships between its components, information must flow mainly from the forward end toward the rear. Osteoclasts need to "know" in what direction and how quickly they should resorb, and osteoblasts need to "know" the magnitude of the task they have been set by osteoclasts. Precursor cells of both osteoclasts and osteoblasts need to "know" rather precisely where they should go, since their destinations are not far apart.

The origin, nature, and modes of transmission of the positional information needed to control the direction of advance of a BMU are unknown. There are nerves in bone that enter at the surface and terminate at a lacuna [41], but neither the full extent of their distribution nor their function are known. For hemi-osteonal remodeling, the lining cells that must continually accommodate the advancing osteoclasts are obvious candidates for the transmitter (Fig. 6). For osteonal remodeling, chemical or electrical signals conveyed by the osteocytes and their cell processes within the lacunar-canalicular system, could direct osteoclasts along the right path [2,16]. How preosteoclasts find their way is unknown; the signals could be the same as in activation of remodeling [14,16], or could be unique to an established osteoclast population, and possibly released during apoptosis. Resorption presumably ceases when no more bone in the vicinity of the BMU needs to be replaced, but how this is recognized and communicated remains a mystery. Osteoclasts could be inhibited by prostanoids, released by osteocytes [14,16], but definitive termination is more likely achieved by turning off the local supply of preosteoclasts, which would be the first step in the eventual disappearance of the BMU [22].

By whatever means osteoclasts and preosteoclasts are guided, a mechanism is needed to ensure that osteoblasts follow in their wake. A popular and plausible notion is that growth factors of osteoblast origin, such as TGF-β [42] or IGF-II [43], stored in bone matrix while it was being formed, and released intact during resorption, stimulate the local proliferation of osteoblast precursors [39,40,45], thus relating the number of osteoblasts recruited to the amount of bone resorbed. The same growth factors would likely be released by osteoblasts into the local extracellular fluid; as previously explained, this would not enable more osteoblasts to be recruited during the same remodeling cycle, as has been proposed [46], but growth factors released by each new team of osteoblasts could enhance the recruitment for subsequent teams (Fig. 6).

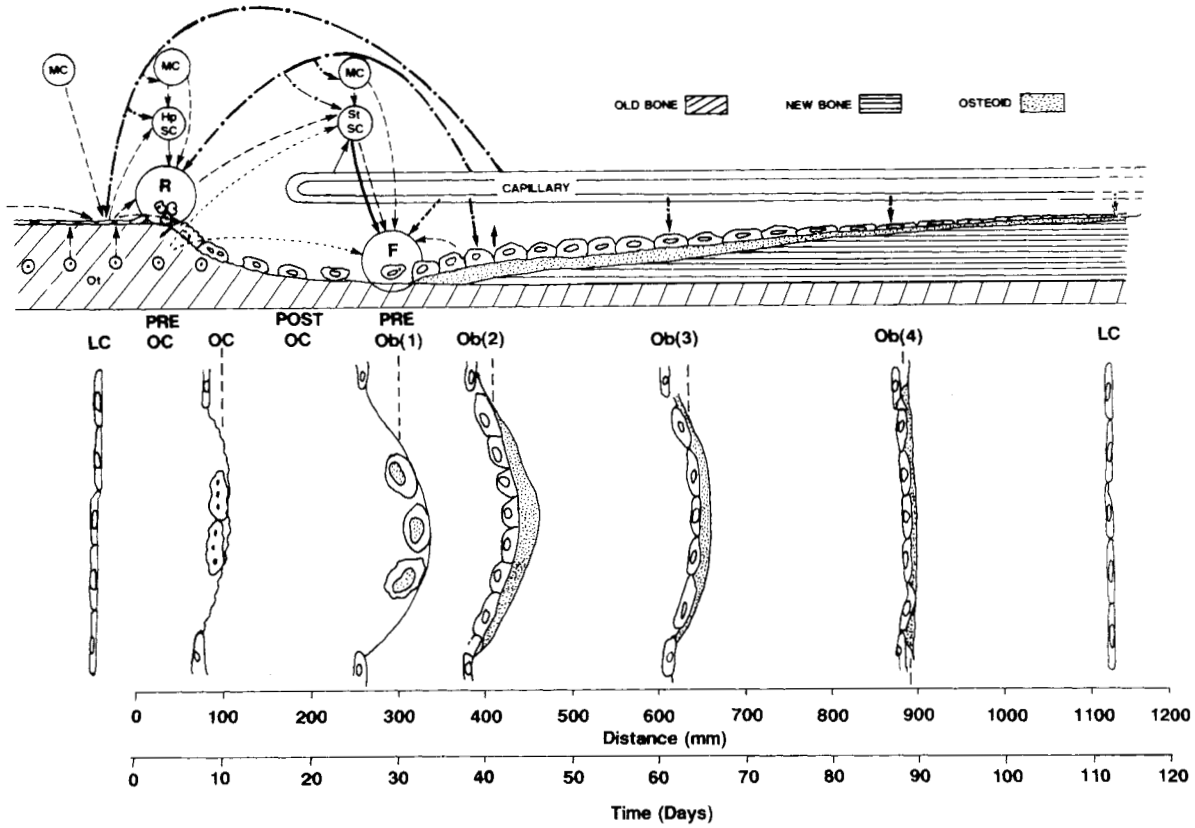

Fig. 6. Hemi-osteonal model of cancellous bone remodeling. A cancellous BMU is depicted in longitudinal section above and selected transverse sections below, which correspond to the sequence shown in Figure 3. The entire structure is traveling from right to left, digging a trench across the surface. The distance and time scales assume longitudinal advance at 10 μm/day; this is based on an estimated total BMU length of 1,200 μm and an estimated time for completion at each cross-sectional location of 120 days. Pathways of cell recruitment and movement are shown as solid arrows. Possible pathways of signal traffic are shown as arrows distinguished according to their origin, whether from other local cells (– – –), via the blood stream (– · –), or bone (· · ·). The connecting lines are located for clarity, not anatomic accuracy. R = region where resorption begins and all new preosteoclasts are recruited. F = region where formation begins and all new preosteoblasts are recruited. LC = lining cell; Ot = osteocyte; Oc = osteoclast; Ob. = osteoblast [numbers refer to stages in Parfitt (27)]; HpSC = hematopoietic stem cell; StSC = stromal stem cell; MC = unspecified marrow cell. For further details, see text.

Although many growth factors are undoubtedly present in bone matrix, that any function in this manner in vivo has yet to be determined; it is conceivable that osteoblast recruitment was already programmed when the need for remodeling was recognized. Substances released from resorbed bone might provide chemotactic signals for osteoblast precursors [3,14], but the new osteoblasts would need an additional signal to assemble in the right place. This could be a substance released by osteoclasts and incorporated into cement substance, such as tartrate-resistant acid phosphatase [44]. When resorption continues without interruption at the same location, as during growth and modeling, no cement plane is formed so that the osteoblasts can be directed elsewhere.

A neglected aspect of BMU physiology is the local micro-circulation. A cortical BMU invariably contains a vascular space, descriptively a sinusoid, presumably connected to both afferent and efferent capillaries (Figs. 1 and 5). Sinusoids have been described close to the surface of cancellous bone [47], but their relationship to BMUs is unclear. In addition to its usual nutritional and metabolic functions, the circulation of the BMU brings precursor cells from their sites of origin, carries protons from sites of bone formation to sites of bone resorption and calcium ions and other constituents of bone mineral in the reverse direction, transports systemic regulatory molecules, and facilitates the local distribution of growth factors and cytokines (Fig. 6). But blood vessels are important not only as conduits,

but as a reservoir of endothelial cells [47,48] and as a source for their products such as endothelin [48,49]. It was proposed many years ago that endothelial cells could give rise to osteoblast precursors during endochondral ossification [50]. A similar role for them in hyperparathyroid bone disease has been suggested [47], but whether endothelial cells contribute to osteoblast recruitment during normal bone remodeling has not been studied. Nevertheless, there is active interest both in the local effects of endothelin on bone and in the mechanisms whereby endothelial cells could direct circulating cells of various kinds toward sites of bone remodeling [48,49].

The preceding discussion sketches very briefly how a conceptual model of the BMU can provide a standard for judging the relevance of in vitro experimentation to in vivo physiology. But although this may limit the range of possible mechanisms that need to be considered, it cannot by itself decide which ones are correct. What must be done is to characterize gene expression, in as much detail as is needed, in cells examined in the same spatial and temporal context as they normally inhabit, using in situ hybridization for specific mRNA molecules and immunocytochemistry for specific proteins. The most informative probes would be chosen on the basis of relevant in vitro work [39] and the characterization of human bone samples using PCR [40]. This approach, which I have termed "molecular histomorphometry," was recently featured for the first time at an international congress [51]; it faces formidable technical difficulties, including the hardness and rigidity of the adult bone in which remodeling occurs, and the frequent failure of the methods to work in the undemineralized sections needed to preserve the kinetic information provided by tetracycline labeling. Even when these difficulties have been overcome, a further hurdle will be the necessity of returning to the study of cortical bone in large animal models, such as the dog or the mini-pig, in which remodeling is fundamentally similar to that in human subjects. Only for osteonal remodeling will it be possible to relate the molecular characterization of individual cells to the spatial and temporal organization of the BMU [52], and only then will the physiologic regulation of normal bone remodeling, at the cellular and molecular levels, be amenable to experimental investigation.

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