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Stephen M. Krane

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Stephen M. Krane, MD*

Ed. Note - This overview was originally presented at the International Symposium on Clinical Disorders of Bone and Mineral Metabolism, May 9-13, 1983. The following list indicates the presentations given in this session at the Symposium and the contents of the corresponding chapter in the Proceedings of the Symposium published by Excerpta Medica. The numbers in parentheses refer to pages in this volume. Complete information about the contents of the Proceedings can be found at the back of this issue.

Tracer measurements of bone remodeling and their significance in involutional osteoporosis. J. Reeve, M. Arlot, R. Hesp, M. Tellez, and P.J. Meunier (99)

Excretion of urinary hydroxyproline peptides in the assessment of bone collagen deposition and resorption. K.I. Kivirikko (105)

Procollagen extension peptides as markers of collagen synthesis. L.S. Simon and S.M. Krane (108)

Essential to understanding the pathophysiology of skeletal diseases and evaluating the results of therapy are markers for bone cell function and a means to accurately measure the synthesis and degradation of the extracellular matrix of bone. As knowledge has advanced concerning the structure and composition of bone and pathways of biosynthesis of the skeletal components, new markers have become available. The utility of techniques that have been used for years to quantitate bone formation and resorption now have a different perspective.

Bone is a two phase material composed of an inorganic mineral phase (≈65% of weight) and an organic matrix (≈35% of weight). The calcium-phosphorus mineral phase, at least part of which is organized with respect to the collagenous portion of the organic matrix, consists of poorly crystalline hydroxyapatite (1). The composition of the organic matrix of bone is described in Table I.

The collagen of bone is almost exclusively type I, identical in primary structure to the type I collagen in other organs (2). However, there are differences in certain posttranslational modifications between the type I collagens in skin and bone, particularly with respect to content of hydroxylysine, hydroxylysine glycosides, and the hydroxylysine-derived hydroxypyridinium crosslinks (3). The distinctive collagenous product of the osteoblast can thus be ascribed to the expression of genes coding for proteins other than the component α1(I) and α2(I) chains.

Over the past several years, several noncollagenous proteins unique to bone have been identified and characterized. The isolation of the α-carboxyglutamic acid (Gla) protein (osteocalcin), osteonectin, and bone proteoglycan has been made possible by avoiding artifacts introduced by mechanical shearing forces that cause release of proteases during demineralization and extraction. Osteonectin is a good example (4,5). It represents more

TABLE I

Composition of the Organic Matrix of Bone

I. Type I collagen: 90-95% of total

II. Noncollagenous proteins

A. "True" matrix constituents (osteoblast-derived)
1. Osteonectin
2. Bone sialoprotein
3. Bone proteoglycan
4. Other glycoproteins
5. Phosphoproteins

B. Bound to mineral phase (osteoblast-derived)
1. Bone Gla protein (osteocalcin)

C. Serum-derived
1. α_2-HS glycoprotein
2. Other serum proteins

*Department of Medicine, Harvard Medical School and Massachusetts General Hospital, Boston

Address reprint requests to Dr. Krane, Massachusetts General Hospital, 32 Fruit Street, Boston, MA 02114.

than 25% of the noncollagenous proteins of bovine bone. Osteonection, which is made by osteoblasts, may have a functional as well as structural role in regulating collagen and protein synthesis. Radioimmunoassays developed for this protein should serve as another marker for matrix protein metabolism in humans.

In the process of bone formation, mineral ions enter the inorganic phase and are removed in the process of bone resorption. Thus, the kinetics of radiocalcium and radiostrontrium uptake by the skeleton provide a means to quantitate bone formation and resorption. Validation of the derived rates is best accomplished by employing an independent method. In this Symposium, Reeve and associates (pp. 99 ff.) have described histomorphometric methods with double tetracycline labeling to calculate local bone formation rates. These correlate with whole body radiotracer data that are properly corrected for long-term exchange processes.

As suggested in Fig. 1, the synthetic activities of osteoblasts might be monitored without direct bone sampling if byproducts of the matrix components that become part of bone were released quantitatively and easily measured. Moreover, the release of degradation products of bone resorption could provide an accurate index of these processes. Considerable effort has been devoted to the measurements of collagen-related markers. Many investigators have relied on measurement of urinary hydroxyproline excretion to evaluate metabolic bone diseases. Unfortunately, total urinary hydroxyproline represents only 10-20% of that released from collagen degradation, since free hydroxyproline released from collagen-derived oligopeptides is oxidized to a number of other products. In individuals who excrete relatively low amounts of urinary hydroxyproline, e.g., osteoporotics, a minor change in catabolism resulting from therapy could produce major changes in hydroxyproline excretion. Furthermore, significant amounts of urinary hydroxyproline are derived from collagen sequences other than bone, particularly in those individuals excreting less than 40 mg/day. For these reasons, other measurements have been used. Measurement of hydroxylysine and its glycosides more accurately reflects the amount and source of collagen degradation. Although these methods are tedious, they are constantly being modified. Kivirikko's review (pp. 105 ff.) discussed the measurement of serum levels of posttranslational collagen-modifying enzymes in disease states.

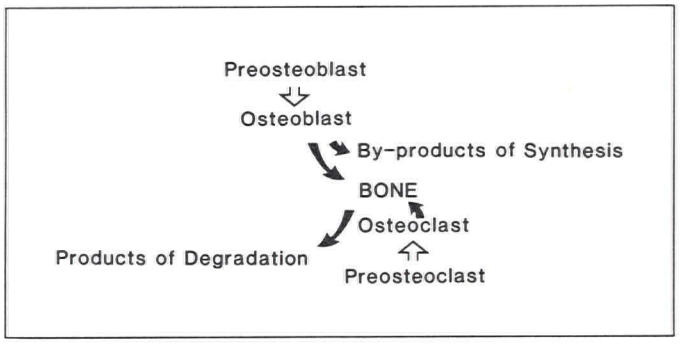

Fig. 1

Schematic Representation of Bone Formation and Resorption.

Collagens are synthesized as procollagens, which are molecules with extra sequences at either end that must be cleaved when the mature fibril is formed extracellularly. Simon and I (pp. 108 ff.) discussed the use of radioimmunoassays for two of the procollagen extensions to evaluate changes in Paget's disease and other conditions. The procollagen extensions are examples of byproducts of synthesis (Fig. 1). The aminoterminal peptides may have a regulatory role in the feedback control of collagen formation.

A radioimmunoassay of bone Gla protein has recently been developed to detect markers for the noncollagenous proteins of bone. This 49-amino acid polypeptide is produced by osteoblasts, and its rate of synthesis is subject to endocrine control. Probably synthesized at some stage in bone formation later than that of noncollagenous proteins such as osteonectin, its role in bone remains to be defined. Nevertheless, this Gla protein is unique to bone and circulates in normal human plasma. Moreover, as discussed by Deftos and Catherwood (pp. 112 ff.) circulating levels change in bone diseases and in response to alterations in parathyroid hormone secretion. Whether levels of bone Gla protein reflect bone formation primarily or resorption remains to be determined.

Several noncollagenous proteins of bone that are not produced by bone cells, such as albumin and α_2-HS glycoprotein, become part of the extracellular matrix in the course of bone formation. The α_2-HS glycoprotein is synthesized in the liver, released into the circulation, and selectively taken up by bone. Thus, levels of this protein in plasma might reflect inversely the rate of bone formation. Smith, et al (pp. 116 ff.) discussed their results using a radioimmunoassay for α_2-HS glycoprotein in patients with metabolic bone diseases. The direction of change in levels of the protein was as predicted, but the change was not great enough for the measurement to be widely adopted.

To quantitate bone formation, it would be highly desirable to have a measurement that reflects osteoblastic activity directly. Measurements of circulating alkaline

phosphatase activity have been available for over 50 years. Bone is rich in this enzyme, which is localized predominantly in osteoblasts. Despite this evidence, the role of alkaline phosphatase in bone formation has yet to be defined. As discussed by Whyte (pp. 120 ff.), the enzyme in bone can be distinguished from that in other tissues by immunological and kinetic methods.

Considerable progress has been made to develop markers for bone formation and resorption that do not depend on bone biopsy. In the future, many methods for measuring these markers, which are currently being developed, should provide accurate, quantitative information for diagnosis, understanding the pathophysiology of metabolic bone diseases, and evaluation of therapy.

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