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AGE CHANGES IN HUMAN BONE: AN OVERVIEW

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That regular decay of nature which is called old age, is attended with changes which are easily detected in the dead body, and one of the principal of these is found in the bones, for they become thin in their shell and spongy in their texture.

-- Sir Astley Cooper, 1824.

AGE CHANGES IN HUMAN BONE: AN OVERVIEW

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Anyone who reviews microscopic sections of bone taken from patients fifty or older notices subtle changes which occur consistently enough to permit fairly accurate estimation of the patients' ages. The bone is porous with plugged haversian canals and canaliculi; many osteons are incompletely mineralized, hypermineralized or hypomineralized; and empty osteocyte lacunae increase ^{in number.} The lumina of haversian canals are often larger than normal and their blood vessels appear sclerotic. The proportion of the skeleton composed of lamellar bone increases and microinfractures (which may seem to be artifacts but really aren't) are noted in areas of strain and contribute to the death of interstitial bone. After sixty, the balance between osteoblastic and osteoclastic activity has shifted to result in increased numbers of imperfectly resorbed osteons which persist as interstitial fragments and, most notably in the subendosteal areas, recently formed osteons have larger, often marrow-containing, lumina to impart a trabecular pattern to this area which the observer may call "osteoporosis". These changes, in Putschar's words (1, p. 401), ". . . blend into pathological forms of osteoporosis which either precede them in time or exceed them in degree or both." Yet, in Dequeker's view:² "Bone involution seems to be a general phenomenon and a normal manifestation of the atrophy of tissues in the process of ageing".

Cellular Aging

Martin³ reviewed cellular aging and defined senescence as "a constellation of deteriorative changes in structure and function of an organism, generally occurring after sexual maturation, which results in a progressive decline in the efficiency of homeostasis and in the success of the reaction to injury."

Tissue cultures, largely of skin fibroblasts and for that reason to be interpreted cautiously, because adult post-mitotic cells live out their lives in a matrix of other cells and cell types, suggest that large populations of normal cells and clones *of cells eventually cease replication and that relative rates* of clonal attenuation may be quite significant in the whole problem of cellular senescence. Unlike transformed cells -- the HeLa cell is best known and most extensively studied -- diploid somatic cells have finite growth potentials in tissue culture, approximating 50 ± 10 population doublings. These non-neoplastic, non-transformed cell colonies grow old and die. Martin³ cautions that "hot spots" and "cold spots", collections of more and less growable cells, exist in biopsy material, but that the percentage of clonable cells of many varieties consistently declines with the donor's age. Possibly clonal senescence is a two stage process as growth first slows and then true senescence occurs at varying rates in postreplicative cells.

Hormonal Influences

Rasmussen and Bordier⁸ note that hormonal and ionic factors contribute to modulation of osteocytes to bone-resorbing and bone-forming cells. Ordinarily, these osteocytic functional fluctuations affect only the tiny volumes of lacunar and canalicular bone surrounding the osteocyte and its extensions. These particular compartments of metabolically labile bone ordinarily surround the rather small percentage of the total osteocytes in an area engaged in osteocytic osteolysis.⁵ Courpron, Meunier et al.⁶ argue for an individual bony personality (personnalité osseus individuelle) and that bone aging involves a parallel involution of both the bone and hematopoietic cell lines.

Dequeker² reports that estrogens inhibit bone resorption in mammals, possibly by reducing the parathormone effect on bone, and suggests that the postmenopausal increase in serum calcium levels and alkaline phosphatase activity

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comes from diminution of estrogenic protection against the bone resorbing action of parathormone, which would also explain the increase in serum calcium levels which occurs among men past sixty-five.

'Clasts, 'Blasts and 'Cytes

Histologically, new bone is deposited only at the sites of previous bone resorption. When calcitonin is administered to young rabbits, the number of multinucleated osteoclasts present at resorption surfaces decreases within fifteen minutes. They are replaced by mononucleated cells which seem to be preosteoblasts and which give rise to osteoblasts, so that the osteoclasts did not die but modulated into osteoblasts. The osteoblasts and pre-osteoblasts did not undergo mitosis.⁴ Harold Frost's^{7,8} brilliant studies over many years have defined a "normal sequence" of events in endosteal bone remodeling: activation of a new group of cells, resorption of bone by the daughters of these cells, and sequential formation of new bone at the site of previous resorption. Osteoclasts and osteoblasts therefore do not constitute separate cell pools but represent differing functional states of the same cells:

Osteopenic bone typically does not have more osteoclasts than normal, but does have increased resorptive surface areas and decreased bone-forming surface areas. Osteoprogenitor cells in older people form osteoclasts with fewer than the normal number of nuclei and after fission, modulation may be

delayed or some of the modulated cells may fail to develop into functional osteoblasts. Inactive or arrested resorption surface area increases, active bone-forming surface area decreases and for these reasons, Rasmussen and Bordier⁴ attribute osteoporosis to osteoblast failure. The percentage of Howship's lacunae which contain osteoclasts decreases up to the fifth decade, slightly increases thereafter, and the percentage of empty osteoclastic lacunae is minimal between 30 and 40.⁹ Delling⁹ describes certain age-related changes on the inner surfaces of bone: after the fifth decade, the percentage of large and presumably bone-resorbing osteocytes increases and that of small and presumably bone-forming osteocytes falls, as empty osteocyte lacunae increase with advancing years.

Collagen

Schmidt, Kalbe and Sielaff¹⁰ state that with advancing age, soluble bone collagen fractions and metabolism decrease only slightly and the collagen half-life increases proportionately. Both the insoluble bone collagen half-life and the insoluble share of the total bone collagen increase, but its metabolic rate decreases. This shift in the proportions of soluble and insoluble bone collagen and increased half-lives are apparently associated with collagen fiber interlacing by cross-linking and hardening, and corresponds to what is known of other connective tissue collagens with aging. Bone collagen is not inert even in old age, but with aging, insoluble collagen is withdrawn from resorption and therefore from metabolism. The elimination of hydroxyproline, and especially of free hydroxyproline, is reduced by about half from the third to the seventh decade and is related to reduction in the soluble collagen fraction. Whether apolarly attacking collagen peptidases also undergo an age-associated decrease is not known.

Fujii, Kuboki and Sasaki¹¹ report a marked association with age in the decrease of dihydroxylysinoxynorleucine and hydroxylysinoxynorleucine with significantly increased lysinoxynorleucine in both bone and articular cartilage collagen. Simultaneously, the collagen crosslink precursors hexitollysine and dihydroxynorleucine increased. They suggest that both collagens become less soluble with age by in vivo reduction of crosslinks or transformation to unknown forms of non-reducible linkages.

Grant and Prockop¹² suggest that the process of complete crosslinking of collagen may require months or years, and that the duration of this process and stiffening of connective tissues with age raise the possibility that increased crosslinking of collagen may be critical in aging, although no evidence yet suggests that inhibiting this crosslinkage retards the effects of aging. Dequeker² concurs that with advancing age, no striking qualitative changes occur in the collagen or mineral composition of bone, although bone collagen saline solubility decreases with age, probably because of the increased numbers of collagen crosslinkages.

Ground Substance

Schmidt, Kalbe and Sielaff¹⁰ note that proteoglycane synthesis in the ground substance of bone is reduced with age apace generally reduced rates of bone protein synthesis and rapidly decreased sulphate radical incorporation. With aging, the hexosamine and uronic acid content and hexosamine-hydroxyproline ratio decrease, proteoglycane metabolism decreases and hydroxyproline content remains the same or very slightly increases. Not only do the metabolic rates of osteoblasts and osteoclasts decline with age, but deposition of hydroxyapatite in the bone collagenous matrix depends largely on the chemical state of the interstitial substance, especially its proteoglycane content and pattern. These changes lead to a bone matrix inherently less and less mineralizable.

Osteoid

Ultrastructural examination of the bone matrix layer between the osteoblast and the mineralized bone, called osteoid, demonstrates that osteoid is a structured sequential modification of collagen fibrils and mucopolysaccharides, and that it undergoes modification in certain abnormal states.¹³ Whereas fibroblasts form collagen fibers all around themselves,⁹ osteoblasts characteristically secrete collagen precursors and ground substance in only one direction, the collagen precursors passing through the osteoblast cell membrane and, external to the osteoblast, polymerizing with ground substance to yield osteoid.

The calcium concentration within the osteoid seam increases from the region of osteoid nearest the marrow to that nearest the fully mineralized bone, and hydroxyapatite forms along the mineralization front. Inspection of actively forming bone reveals some areas of osteoid covered by large, hexagonal or cuboidal osteoblasts, deep to which rapid primary mineralization takes place, but other areas where the osteoid is covered by flat, resting osteoblasts where a slower secondary mineralization occurs, apparently independently of osteoblast activity. Most cancellous bone surfaces physiologically lack osteoid seams and Howship's lacunae, and constitute a neutral resting surface bordering the marrow space. Whether this resting surface is completely covered by mesenchymal cells is not yet clear, but these flat cells remain connected to osteocyte processes and seem to be important in the regulation of serum calcium and phosphorus levels.

The osteocyte's high metabolic activity, which includes regulation of bone mineral and water content and exchange with extracellular fluid, is fostered by interconnections between osteocyte processes, osteoblasts and osteoclasts.⁹

The lacunar wall, which consists of collagen fibers, ground substance and bone mineral, aggregates about 20 mm.² for each cubic mm. of cortical bone, but the canalicular surface containing cell processes has an area of about 200 mm.² for the same volume. (Some lacunae have a delicate membranous wall, the function and importance of which remain obscure.) Hydroxyapatite crystals are formed on the collagen fibers in a very regular manner to form an optimal mechanical and metabolic system.

Delling⁹ reports that the percentage of nonmineralized bone in relation to a unit volume of bone tissue remains nearly constant after the first two decades and that although the bone surface covered by osteoid increases with age, osteoid thickness decreases. (Significantly thickened osteoid seams at any age suggest disturbed bone formation.) These seams cover 10 to 25 percent of the total bone surface, are minimal between 40 and 50 and increase with age. Delling notes (9, pp. 136-137): "in contrast to the total extent of seams, the percentage of active seams (osteoid with osteoblasts) decreases from $22.4 \pm 8.1\%$ in the first decade to $0.9 \pm 0.6\%$ in the eighth." The percentage of inactive to total bone surface remains constant from the 10th to the 90th year, although the absolute value decreases as bone mass diminishes.

In contrast to the decrease in active osteoid seams with age, the surface covered by inactive seams (osteoid without osteoblasts) increases from the fifth or sixth decade. Delling⁹ suggests that this is related to renal arteriosclerosis and reduction in available renal parenchyma, the site of hydrolyzation of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol, the active metabolite of Vitamin D for bone tissue. Hence, reduction in the quantity of active osteoid, and its eventual mineralization, may be secondary to physiologic, or pathologic, age changes in other organs, just as the efficiency of gastrointestinal calcium absorption declines with age.

Compact Bone

Kerley¹⁴ described fairly consistent changes in the shafts of long bones associated with advancing age, most marked in the inner third of the cortex with outer third being least affected. Description of these changes requires certain definitions. Osteons are vascular channels surrounded by concentric lamellae containing rather evenly spaced osteocytes and bordered by a peripheral growth reversal line. Fragments are portions of old osteons which may surround the peripheries of newer osteons. The number of osteon fragments increases as more and more osteons are laid down until, in extreme old age, most complete osteons are surrounded by fragments of older osteons. Circumferential lamellar bone consists of evenly spaced, generally parallel or concentric bone lamellae in the outer subperiosteal portion of the cortex, with a collagenous matrix consisting of long, parallel, fairly uniform fibers. Nonhaversian canals contain primary vascular channels formed by incorporation of small, peripheral, originally periosteal vessels into the bone first deposited as the cortex expands. These have no osteonal pattern, lack growth reversal lines and represent unremodeled bone, in contrast to osteonal bone formed by osteoclastic remodeling.

At the tissue level, bone remodeling is continuous.^{7,15} Within lamellar bone, cutting cones produce cavities of the order of 200 micra in diameter and as much as several millimeters long. This "cutting head" of osteoclasts is followed by a fibrocapillary leash, and the cavity cut by the osteoclasts is filled in centripetally by osteoblasts which arise from the capillary perithelial cells or are modulated in situ to produce the secondary osteon. Osteoblasts deposit the bone of secondary osteons in a definite, organized pattern wherein collagen matrix fibers change course in each layer, again leading to maximum flexibility and physical strength. Space between these secondary osteons is occupied by interstitial lamellar bone, and if this interstitial bone cannot be adequately nourished by the canaliculi, it dies.

The relative proportions of dead to living interstitial bone increases steadily with age.

Kerley¹⁴ describes a life cycle for cortical bone. The osteons increase from birth to old age, the proportion of circumferential bone decreases with age, and nonhaversian canals disappear during the sixth decade, indicating a very radical decrease in subperiosteal bone deposition by that time. The earliest phase of bone deposition is rapid formation of radial spicules of coarsely woven bone with irregularly arranged collagen fibers having no particular relationship to the large vascular spaces which they enclose. Later, vascular spaces are occupied by new bone apposed on their walls to become the primary osteons.¹⁶ As the cortex and medulla expand, small subperiosteal blood vessels running along the long axis of the bone are incorporated by the expanding peripheral cortex to form nonhaversian canals. Finally, osteoclastic cutting cones form osteons which are typically themselves partially destroyed by subsequent remodeling.

Pankovich, Simmons and Kulkarni¹⁷ studied microradiographs of rib sections from patients 8 to 80 years old, and found changes in the percentages of osteons with low, intermediate and high mineral density as the skeleton aged. They describe a characteristic osteon of old age as having low to intermediate mineral density, wide haversian canals and hypercalcified borders. They describe a trend toward increasing percentages of osteons with incomplete growth arrest lines in older patients, and suggest that intraosteonal remodeling and growth retardation play increasingly important roles in bone turnover in the aged skeleton, and contribute to the characteristic senescent increased porosity and lower mean osteon mineral density. Intraosteonal remodeling may preferentially affect older osteons because the oldest peripheral lamellae are typically highly mineralized and this process -- which does not seem to effect interstitial bone -- seems to be a mechanism whereby aged osteon mineral density is reduced and skeletal

porosity increased.

Trotter and Hixon¹⁸ examined the weight, density and percentage ash weight of dry, fat-free bony skeletons of both sexes, black and white, from the sixteenth week of gestation to 100 years old, and found a gradual loss in bone weight and density (but not volume) that began during the fourth decade, and averaged 15.6 grams yearly. Change in weight was accompanied by change in ashed weight. Race and sex differences were marked by the second decade: black skeletons exceeded white and male female in mean weight and density and, though to a smaller extent, in percentage ashed weight. Smith, Khairi and Johnston,¹⁹ by longitudinal studies, documented that individuals lose bone mineral at unequal rates with age. Bone mineral content, using the radius as a standard, had a normal statistical distribution at all ages, and variation from the normal did not increase with age, so that because no bimodal distribution emerged, a distinct population of "rapid bone losers" (as some workers suggest exists) could not be identified. Hence, the rate of individual bone mineral loss is not constant, the rate of bone mineral loss is proportionate to the amount of mineral present at maturity, or both. They suggest that the bone mineral content of patients identified as osteoporotic is at the lower end of a normal curve, probably because they have a low bone mineral content from maturity.

Bone Loss

The problem of skeletal aging may be summarized² as decreased skeletal weight added to decreased physical density (weight/volume) of individual bones. Similar changes have been described in palaeopathologic material, so this is not a modern phenomenon. Although good statistical relationships exist between changes in the peripheral and axial skeletons, changes in one particular bone do not accurately predict changes in another. It has been known forever that old people have more fragile bones than young people, and Dequeker² suggests that bone

strength, which measures compliance, and bone brittleness, which measures breakability, may be different things, and old people may have "bad bones" in terms of brittleness.

During adult life, bone is gained or lost at one or any combination of four sites: periosteal surfaces, surfaces of vascular channels within cortical bone, cortical endosteal surfaces and trabecular endosteal surfaces. Even after bones stop growing in length, cancellous and cortical bone undergo constant resorption of existing and deposition of new bone. Changes in this balance underlie every disease affecting the skeleton. Periosteal bone growth is not merely compensation for endosteal bone loss, because subperiosteal deposition in old age fails to keep pace with subendosteal resorption, nor is continuing bone deposition invariably related to weight-bearing or flexion stress.

Loss of bone after the fifth decade comes from three processes: slightly increased osteoclasia which reduces the amount of cancellous bone; simultaneous decrease of osteoblastic activity which accentuates rarefaction by decreasing new bone formation; and delayed mineralization of inactive osteoid seams. (Some osteoblasts thicken main trabeculae in a form of compensatory hypertrophy without affecting the net process.)

The total bone tissue, excluding marrow, constitutes about 20 percent of cancellous bone, a proportion which remains fairly constant until about the fifth decade, after which loss of bone mass occurs as rarefaction of bone is accompanied by larger marrow spaces filled by fat and nests of hematopoietic marrow.⁹

~~(Delling 1973)~~ Dequeker considers that the endosteal cavity expands in response to endosteal bone loss, and that this process accelerates after 50 as the endosteal surfaces enlarge faster than the periosteal surfaces.²

Because bed rest so quickly leads to osteoporosis, Dunnill, Anderson and Whitehead²⁰ studied changes in the bones of patients who died in accidents, and found that the proportion of a bone occupied by asseous tissue decreased steadily with age as, with advancing years, fatty marrow steadily enlarged to replace both bone and hematopoietic marrow. They also agree on an overlap between normal skeletal senescence and osteoporosis. Takahashi and Frost²¹ found that women begin life with significantly less bone than men, primarily because their bones are smaller, that both sexes have maximum amounts of bone at 15 to 15, and lose about a quarter of this maximum by sixty. They found no correlation between a bone's total cross-section and its cortical component from puberty to the menopause, and suggest that if osteoporosis is related to the menopause, the mechanism is extra-ovarian. Increase in total bone cross-section area occurs after sixty, more notably among women than men, and although new bone formation falls to a basal level, it does not cease. The periosteal surfaces are never in negative balance, that is, the space inside the periosteum never shrinks. Smith, Khairi, Norton and Johnston²² found that the mean rate of mineral loss is not constant with aging, is slower in the elderly than during earlier postmenopausal years, and that factors other than decreased physical activity are more significant in determining the rates of mineral loss.

Rasmussen and Bordier⁴ state that slow but progressive bone loss occurs throughout life although the transverse diameters of bones increase from a net positive balance of subperiosteal and a net negative balance of subendosteal bone. From 20 to 50 or 55, women lose bone slightly more rapidly than men, but experience an abrupt loss of skeletal mass between 50 or 55 and 65, after which the rate of loss again stabilizes.

Current views as to the pathophysiology of bone loss may be summarized² as a senescent imbalance between bone deposition and bone resorption in the latter direction. Parathormone plays a major, but indirect, part in enhanced bone resorption by changing end-organ sensitivity, particularly to changes in the androgenic/estrogenic balance. Currently, dietary calcium deficiency is not considered a major factor in bone loss and human beings seem to adapt quite comfortably to low calcium diets, absent pregnancy and lactation which are rather rare geriatric problems. However, a peculiar calcium deficiency may arise from primary or secondary hypercalciuria which lowers the serum ionized calcium content and results in increased parathormone levels and, therefore, increased bone resorption. Frost⁸ has documented that bone formation and resorption tend to change in the same direction with a variable time lag between the two phases, thus, treatment may reduce resorption but the organism will then reduce bone formation to match.

Mechanical Effects

Burstein, Reilly and Martens²³ studied the tension, torsion and compression responses of bone from a population of patients between 21 and 86, and found no significant differences in the mechanical properties of bone between men and women, nor in the tension properties of bone from normal, osteoporotic and adrenocorticosteroid treated individuals. The only consistent difference was that ultimate strain at the point of fracture decreased 8.4 percent for each decade. They suggest (23, p.86):

. . . That this decrease with age was seen in both femoral and tibial bone tissue suggests that the ultimate strain reflects a property or quality which depends on the age of the individual at the time that the bone tissue under test was originally deposited. Hence, age effects on ultimate strain are determined

by the age of the organism rather than the time that the

tissue has been in situ. . .

They attribute declining mechanical strength of bone to changes in the structure of the bone collagen matrix.

Burstein, Zika, Heiple and Klein²⁴ elegantly tested wet bovine haversian bone as though testing the plastic behavior of a metal, and found by sequential decalcification that as bone mineral content decreased the tension yield point and ultimate stress decreased progressively without changing yield strain or ultimate strain short of complete decalcification. Their findings "are consistent with an elastic-perfectly plastic model for the mineral phase of bone tissue in which the mineral contributes the major portion of the tension yield strength". They suggest that collagen itself plays only a minor role in producing bone's tension yield strength. Thus, when a bone is subjected to tensile loading, there is an initial elastic response in which the load deformation curve is essentially reversible, followed by a plastic -- or permanent -- deformation of the bone. Under tensile stress, then, bone mineral deforms plastically and acts as a metal, not as synthetic plastic composites.

Mineralized bone develops numerous tiny cracks and dislocations once a critical mechanical stress is reached and, during stress-induced plastic deformation, these cracks propagate a limited distance in the bone until they are interrupted.²⁴ Added deformation causes additional cracks or dislocations, and once the strain reaches the point that structural interruption no longer can stop the propagation of these microfractures, a "catastrophic crack" spreads and the tissue fails.

Weaver and Chalmers²⁵ studied changes in bone strength with aging, and found that most of the fractures which increase with age occur in bones wherein trabecular bone provides a significant portion of support, and that the compression strength of cancellous bone is closely enough related to bone

mineral content that compression failure strength proves a reliable function of mineral content regardless of age or sex. Bone strength and mineral content diminish steadily with age. Before 45 or 50, there is no sex difference, but because bone loss begins earlier and is more profound in women than in men, by the seventh decade, women's vertebral bone ash weight is 20 percent less than men's. (Activity, of course, influences cancellous bone strength and mineral content, an influence exerted through the orientation and distribution of trabeculae.)

Summary

The human skeleton steadily changes structure and mass during life because of a variety of internal and external factors. Extracellular substance and bone cells get old, characteristic structural remodeling occurs with age and these age-related changes are important in the discrimination between pathological and physiological changes. Perhaps 20 percent of the bone mass is lost between the fourth and the ninth decades, osteoblasts function less efficiently and gradual loss of bone substance is enhanced by delayed mineralization of an increased surface area of thin and relatively less active osteoid seams. After the fifth decade, osteoclasia and the number of Howship's lacunae increase, and with age, the number of large osteolytic osteocytes increases as the number of small osteocytes declines and empty osteocyte lacunae become more common. The result is greater liability to fracture and diminished healing or replacement of injured bone.

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