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EDITORIAL REVIEW

Role of bone in regulation of systemic acid-base balance

The highly specialized hard structure of the bone not only serves as the supportive framework of the body but enters into metabolic interrelationship with it. For many years the skeleton has been considered to be a source of buffer contributing to the maintenance of a stable systemic pH on one hand and participating in the defense against acid-base disorders on the other hand [1–3]. The linkage between bone and the body acid-base status can be described as both a blessing and a curse. While bone performs this buffering process in the defense against major systemic acid-bone perturbations, it is the very same process of buffering which will ultimately lead to dissolution of the bone mineral.

This paper reviews the current information on the mechanisms by which bone contributes to systemic pH regulation. The experimental and clinical evidence for the generation of various forms of bone disease related to the buffering process will also be reviewed.

Since bone responds to acid-base disorders through its various constituents, it would seem appropriate to start this review with a general description of bone composition and microenvironment.

Bone composition

Bone is a specialized connective tissue that consists of a matrix in which various kinds of cells are dispersed.

Figure 1 provides a schematic representation of an individual microscopic bone unit. As can be seen, the bone matrix space is composed of both inorganic (mineral) and organic components. It is the organizational pattern and relationship of these two components which determines the successful mechanical function of the skeleton. The organic, unmineralized matrix, also called osteoid comprises about one-third of the total skeleton weight. The osteoid consists predominantly of type 1 collagen with small amounts of proteoglycan, lipids and several noncollagenous proteins including the recently characterized beta-carboxyglutamic acid containing protein, osteocalcin, as well as fibronectin, osteonectin and many others [4, 5]. The inorganic mineral phase of bone constitutes about two-thirds of the total matrix volume and it mainly consists of crystals of hydroxyapatite. The term apatite implies that the arrangement of the constitutent ions in the lattice structure of the crystals conforms to that of naturally occurring minerals called apatites. The general formula of the apatite minerals is $Ca_{10}(PO_4)_6\ X_2$. This is not a molecular formula but specifies the relative proportions of the smallest number of ions needed for the smallest repeating structural unit of the crystal (Fig. 2). In bone mineral X = OH^- and hence the term hydroxyapatite. The hydroxyapatite crystal is extremely small and is in the order of 200 by 30 to 70 Å. Such tiny crystals present an enormous surface area estimated to be between 100 and 200 square meters per gram of bone [6]. This large surface area renders the bone mineral a quite impure hydroxyapatite since at the time of crystal formation some of its constitutent ions can be replaced by other ions of approximately the same radius, producing minor defects in the shape of the crystals which do not affect the overall structure [6, 7]. Thus, the calcium position can be substituted for by lead, manganese, sodium magnesium and strontium. Carbonate (CO_3^{2-}) can substitute for phosphate or for the hydroxyl ions [7]. The ionic composition of bone mineral is summarized in Table 1 and is based on the data derived by Armstrong and Singer [8]. The skeleton contains 99% of the body's calcium and also 35% of the sodium, 80% of the carbonate, 80% of the citrate and 60% of the body magnesium.

Throughout life, the bone mass is continuously turning over by a well regulated coupling of the processes of bone formation and resorption. During the growth years, formation exceeds resorption and skeletal mass increases. After bone mass reaches its peak, between ages 20 to 30 years, the rate of formation equals the rate of resorption. After the age of 40 to 50 years, resorption exceeds formation and total skeletal mass starts to decrease. The process of bone turnover in adults is known as remodeling. As much as 15% of the total bone mass normally turns over each year in the remodeling process. Bone remodeling is carried out by the two main bone-cell types: the osteoblast and the osteoclast (Fig. 1). The osteoblast is considered to be the cell responsible for the synthesis of the organic matrix and its calcification. It is currently believed that the osteoblast is important also in the process of bone resorption which is ultimately carried out by the osteoclast through its paracrine and endocrine properties (discussed later). Thus, the osteoblast and not the osteoclast bears receptors for bone resorbing hormones like PTH and $1.25(OH)_2D_3$ as well as for cytokines such as interleukin-1 [9–12]. The osteoblast also synthesizes various bone resorbing substances such as interleukin-6 and prostaglandins [13, 14]. The exact mechanism by which the osteoblast transmits its message to the osteoclast, to initiate bone resorption, is not entirely defined at the present. The anatomy of the osteoblast varies from a tall cuboidal to an almost flattened cell about 20 to 30 μm in diameter. It is a mononuclear cell, and in its most active state is rich in endoplasmic reticulum and ribosomes with a well developed Golgi apparatus typical of a "protein factory" cell type. Mitochondria are plentiful and on its exterior plasma membrane it is rich in alkaline phosphatase. The origin of the osteoblasts is most likely from osteoprogenitors among primitive mesenchymal cells [15].

Osteoclasts. These cells are the major cells directly responsible for bone resorption. These are large (20 to 100 μm in

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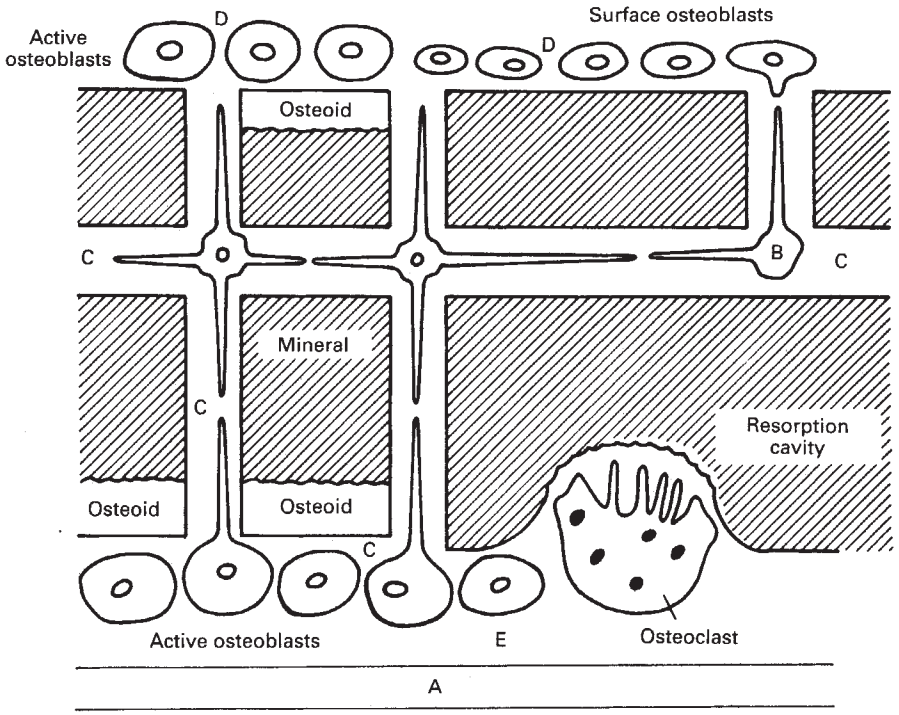

Fig. 1. *Schematic representation of the physiological unit of bone tissue.* Active osteoblasts synthesize the bone matrix which is composed of inorganic (mineral) and organic (osteoid) components. Inactive osteoblasts flatten out over the bone surfaces. The bone area covered by these lining osteoblasts comprises the major part of bone tissue and is quiescent with respect to bone remodeling. Osteoclast is a big multinucleated cell which is primarily involved in digesting bone tissue. **(A)** Vascular space. **(B)** Osteocyte. This cell communicates through protoplasmic extensions with other osteocytes and with osteoblasts. **(C)** Bone canaliculi. **(D)** Gap junctions between adjacent osteoblasts. **(E)** Connective tissue between blood vessel and osteoblast layer. Bone water is composed of two compartments; the water bound to the mineral and osteoid together with the fluid flowing in C comprise the bone extracellular fluid. Fluid in A and E comprise the systemic extracellular fluid. The lining osteoblast layer might serve as a membrane which separates between the two compartments. Compartmentalization of bone ECF is strongly suggested by the existing ionic gradients between bone ECF and systemic ECF.

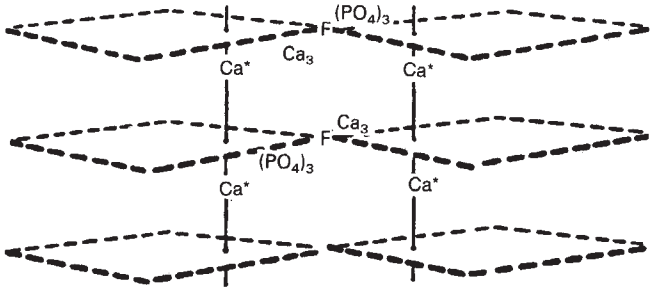

Fig. 2. *A shorthand representation of the fluorapatite unit cell.* In hydroxyapatite crystal, the fluoride (F) ion was replaced by OH^-. This abbreviated representation clearly differentiated between the triple or "screw axis" calcium positions arranged spirally around the fluoride ions and the columnar calcium positions designated by *. (Adapted from Ref. 6).

Table 1. Ionic composition of bone mineral

Cation		Anion	
Ca^{2+}	6.66	PO_3^-	4.02
Mg^{2+}	0.18	CO_3^{2-}	0.79
Na^+	0.32	$Citrate^{3-}$	0.05
K^+	0.02	Cl^-	0.02

Data are expressed as mmol/g of dry fat free bone. Taken from Ref. 8.

diameter) cells of bone-marrow hematopoietic mononuclear cell origin that fuse into multinucleated cells [16, 17]. During bone resorption, the cytoplasmic membrane of the osteoclast is thrown into deep folds and clefts called a ruffled border, increasing the cell and bone surface available for lytic activity. As mentioned, bone resorption and formation are closely coordinated processes. Thus, resorption cavity created by the osteoclast is often the site of subsequent osteoblastic activity which fills in the cavity with new bone [18]. A single turnover cycle in a given bone unit, as characterized by coupled osteoclastic resorption and osteoblastic formation may last for approximately eight months [19].

As the osteoblast becomes less active in matrix formation it flattens out, its alkaline phosphatase activity declines as does the basophilic nature of its cytoplasm, and it becomes embedded within the mineralized matrix. It is now called an osteocyte. This cell occupies small lacunae within the bone substance and communicates through protoplasmic extensions with neighboring cells through the bone canaliculi. (Fig. 1). Osteocytes in their lacunae are surrounded by mature bone and in spite of not dividing, they contribute to dissolution of mineral in their immediate vicinity. Other inactive osteoblasts, flatten out over the surface of bone and will be in contact with the osteocytes via syncytial cell processes lying in bone canaliculi as diagrammatically depicted in Figure 1. The bone surfaces covered by these lining osteoblasts occupy about 80% of the skeleton and they are quiescent with respect to bone remodeling, namely they do not participate in active bone formation and bone resorption [20]. Interestingly, however, the quiescent surfaces can be shown by autoradiography to take up radioactive calcium ions [21] which in the absence of net addition of bone, must be balanced by loss of the same number of unlabeled calcium ion. Based on rates of calcium fluxes related to bone remodeling (formation-resorption cycles) and the rapidity by which the skeleton can correct for deviations in serum calcium (hypo- or hypercalcemia), it is now clear that the instantenous moment to moment regulation of serum calcium is handled by movement of calcium across the large quiescent surface area and not by the active remodeling mechanisms [20, 22]. This is a rapid process and of relatively large magnitude (50 to 100 mmol of calcium daily). Solubilization of mineral at the pericanalicu-

lar zone, probably by osteocytes, releases calcium which is transferred from the interior to the exterior of the bone units and thence into the plasma [23–25]. The bone remodeling system on the other hand, is responsible for movement of bone mineral between bone and body fluids during the active processes of osteoclastic bone destruction and osteoblastic bone formation. This process is slow, and of relatively small magnitude (5 to 10 mmol of calcium daily). It is apparent that while sustained gains or losses of total bone calcium are usually mediated only by an imbalance in the remodeling system, the latter has very little, if any, effect on the short term homeostasis of extracellular calcium. Were it not for the instantaneous homeostatic mechanism, one would expect life threatening increases or decreases in serum calcium in response to the addition to, or removal of calcium from the extracellular fluid.

It is clearly shown in Figure 1 that aside from the large matrix component, the bone also contains a distinct vascular space represented by permeating blood sinusoids and small capillaries. In fact, the most important factor governing density of whole bone specimens is the amount of vascular space in relation to the amount of bone matrix space [26]. The vascular space together with the extravascular compartment (the space between the blood vessels and the lining osteoblasts) comprise the systemic extracellular fluid (ECF) which bathes one side of the osteoblast "membrane" (Fig. 1). On its antisystemic ECF side, the osteoblast is in intimate contact with the osteocytes through their cytoplasmic extensions in the canaliculi. The fluid that flows inside these canalilculi and in the osteocyte lacunae, as well as fluid that is bound to the bone mineral and collagen fibrils constitutes a subdivision of the extracellular fluid and is called bone ECF (Fig. 1) [27].

Several lines of evidence suggest that bone ECF is compartmentalized [28–38]. The physiological meaning of this phenomenon is dual. It first means that bone fluids are not in equilibrium with the systemic fluid and secondly, it implies that an anatomical or functional cellular membrane exists which by separating bone from systemic ECF probably controls the fluxes of ions to and from the bone substance. A strong argument in favor of the existence of a partition between the two compartments by a putative membrane has been provided by the observation of a marked excess of potassium in the bone ECF relative to the systemic ECF [29, 39]. Potassium concentration in bone water may be as high as twenty-five times that of the general extracellular fluids [40]. Although some potassium, usually 20% or less, has to be ascribed to intracellular fluid, 80% or more of the potassium in bone has to be assigned to the bone extracellular fluid [31]. In fact, as an ion being confined only to the bone water compartment, potassium is not incorporated into the mineral phase, nor is it bound to collagen, and therefore it is readily and completely exchangeable with the potassium of the systemic extracellular fluid [39].

The potassium disequilibrium notwithstanding, definitive proof for the existence of bone ECF compartmentalization would require direct sampling of the bone fluid which at the present is not available. However, strong data have been provided by indirect approaches. Neuman and Ramp [22] used a powdered cortical veal bone which was equilibrated many times with buffers of different compositions. The buffer that did not significantly change in composition when exposed to the fresh bone powder was assumed to approximate the average composition of bone fluid. The results obtained by this procedure are summarized in Table 2.

Table 2. Comparison of systemic extracellular fluid (plasma) with bone ECF

Material	Electrolyte content, *mM*					
	Ca	Mg	K	Na	Pi	Cl
Plasma	1.5	0.7	4	140	1.8	100
Bone "fluid"	0.48	0.4	25	125	1.8	130

Source: Ref. 22.

If this is a valid procedure it means that $[Ca^{++}]$ in bone ECF is less than in serum. The Ca:P product is much less than serum, the [K] is much higher and the [Na] and [Mg] are lower. These data, when taken together with the formerly described scheme for short term homeostasis of serum calcium, suggest that whenever calcium moves from bone towards the systemic circulation, this is done by an outwardly directed "pump" located in the outer membrane of the lining osteoblasts. This putative pump will be responsible for the maintenance of the electrochemical gradient across the osteoblast cell layer.

The fact that there exists an ionic gradient between two compartments of the extracellular fluid (systemic vs. bone) separated by lining osteoblasts would suggest that the osteoblast possesses some kind of transporting epithelial cell-like properties. Consistent with this concept is the formation of gap junctions between processes of adjacent osteoblasts as well as gap junctions between lining osteoblasts and the deeper osteocytes [30] (Fig. 1). Such an arrangement permits cell-cell communication as described by Loewenstein [41] and is suggestive of a functional cellular syncytium. That this definite cell-cell complex can handle a vectorial transport is also supported by the specific morphological changes of the lining osteoblasts induced by parathyroid hormone [30]. Upon stimulation of these cells with PTH an increased surface membrane activity takes place which is characterized by the appearance of membrane projections, microvilli and blebs. These changes are similar to those observed in classical transporting epithelia, such as renal collecting duct cells, in response to vasopressin, a hormone known to stimulate solute and water movement and therefore lend further credence to the concept of the osteoblast being a transporting cell. In order for a cell to possess vectorial transport properties, it needs to be polar. In fact, preliminary data from our laboratory as well as by others have demonstrated cell polarity with regards to active membrane calcium pump [42] as well as the location of Na^+/K^+ pump, Na^+/H^+ and Cl^-/HCO_3^- exchangers (unpublished observation).

Overall, further studies are needed in order to shed more light on the concept of a "bone membrane". Specifically, it is unclear what the "bone membrane" is in anatomical terms. The available data, however, permit a visualization of the physiological compartmentalization between the systemic and bone extracellular fluid. This phenomenon may govern the rate of ionic fluxes into and out of the skeleton and probably plays a role in the buffering mechanisms of bone in systemic acid-base disorders.

Surface chemistry of the bone crystal

From its initial seeding induction through its growth maturation and dissolution, the tiny hydroxyapatite crystal must

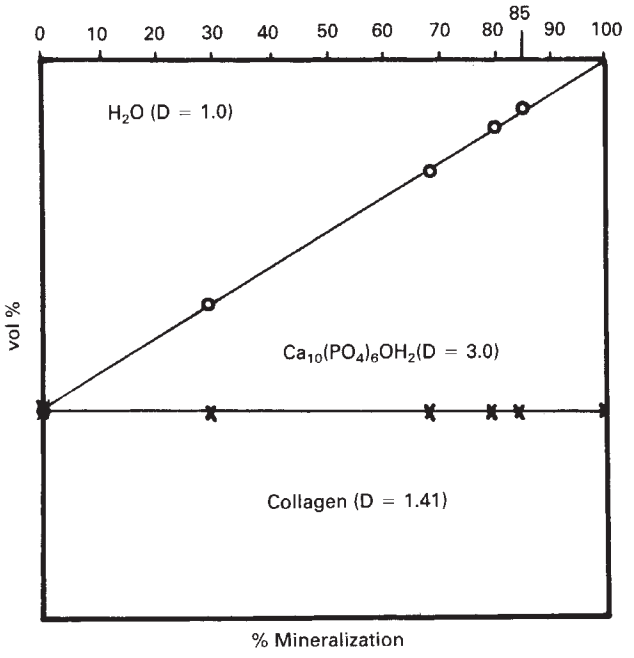

Fig. 3. *The volume relationship existing between the three major components of bone matrix as that matrix becomes progressively mineralized.* Bone mineralization never became 100% or absolutely complete but usually stopped at a point called full mineralization, that is, about 80 to 85% of theoretically complete mineralization. At this point, the small amount of water that was trapped or bound in the bone matrix, no longer transported charged ions to the crystal surfaces. It should be noted that organic bone matrix, once formed by the osteoblast, did not apparently shrink or expand during the mineralization process. D = density and it reflects the water content of a given tissue. (Adapted from Ref. 44)

interact with the water in the bone matrix. The interaction of the crystal with its fluid environment is rather complex. For the crystal to grow, ions must diffuse in from the circulation (systemic ECF). Thus, bone ECF serves as a bridge between crystal surfaces and the systemic extracellular space for the transport of charged molecules. However, when bone mineral is laid down in the process of mineral growth, it displaces an equal volume of matrix water. Therefore the water spaces between crystals or between crystals and adjacent collagen fibers become smaller and smaller to the point where ions can no longer diffuse at appreciable rates [43] (Fig. 3). Since crystals cannot continue growing if ions cannot diffuse in from the outside environment, this also means that mineralization rate should continue maximally up to a certain critical water content. In fact, bone mineralization never becomes 100% or absolutely complete but usually stops at about 80 to 85% of theoretically complete mineralization [44]. It also follows that the age of the bone is the primary determinant of its chemical reactivity and its water content [45, 46]. It is apparent that in young bone compared to old bone, the water content is higher and there is a constant exchange of ions between the hydroxyapatite crystal and its environment. The implication of the phenomenon to our subject is obvious. Bone may actively participate in buffering systemic acid-base disorders while it is actively growing (that is, a young age). In contrast, in the adult skeleton where about 80% of all free bone surfaces are quiescent with respect to remodeling, and less fluid is available for ionic exchange processes to take place, there is probably a much less active role of bone in acid-base homeostasis.

Various methods for measuring bone water have been tried, none of which seemed to be optimal. Thus, values for water content obtained by drying whole bones have little meaning if care is not exercised to completely exclude marrow and other soft tissues from the samples to be analyzed. Also, measuring bone water compartments by using the markers usually employed to measure total water (such as urea deuterium) or extracellular water (such as sucrose, inulin, EDTA) is hampered by physiochemical interactions of these substances with the bone crystal and matrix components [47]. With these limitations in mind, the data by Robinson and Elliot [48, 49] indicate that the approximate values of water in bone obtained from young dogs ranges from 10 to 22% in cortical bone and 30 to 52% in cancellous bone. As the bone tissue increasingly mineralizes, the percentage of total water per given volume of bone decreases. They also defined the specific water spaces in the bone: marrow-vascular, osteocytic and osteoid. Of the total water content in bone, the intracellular compartment constitutes about 20%. The rest, comprising the extracellular fluid, is subdivided into systemic ECF which consists of the vascular space and the space between blood vessels and osteoblasts and the bone ECF which include the osteocytic lacunae, canaliculi and matrix water (Fig. 1). In the matrix, water is bound both to the collagen fibrils [50, 51] and to the hydroxyapatite crystals. The latter fraction of bone ECF, also called the "hydration shell" constitutes the major bulk of bone ECF.

The hydration shell

For many years physicists have accepted the view that a solid surface, when exposed to a liquid, takes on an extremely thin film of bound solvent. The thickness of this film is not known with certainty but hovers around an estimated mean of about 100 Å. The surfaces of hydroxyapatite crystals, too, take on a solvent layer and because the specific surface of these crystals is so large, the total amount of solvent bound can be very high. The physical force that creates this solvent layer depends on the electric charge asymmetry at the crystal-solution interface. As diagrammatically illustrated in Figure 4, in the crystal interior each cation is completely surrounded by a restraining field of anions, and conversely each anion is surrounded by cations. At the surface, however, the ions are not completely shielded. Rather, there is an interface of residual electric charge from each surface ion projected into space (or into the solvent) as a mosaic patchwork of positive and negative fields. This charge asymmetry can be diluted out by forming successive layers of polarized ions which gradually dilute the surface electric asymmetry. The polarized ions usually consist of phosphate ions which are naturally polarizable and of hydrated calcium ions. Ionic calcium in the unhydrated, crystalline state is non polarizable but is quite polarizable by virtue of its layer of oriented water molecules in the hydrated state ($Ca^{++} \cdot 10\ H_2O$). This interface of hydrated calcium and polarized phosphate ions is pictured diagrammatically in Figure 5.

It is clear that in order to fully accomplish the maintenance of electroneutrality, the ions in the hydration shell are not fixed and motionless but are mobile and reactive; only the electrostatic field which is responsible for the origin of the hydration

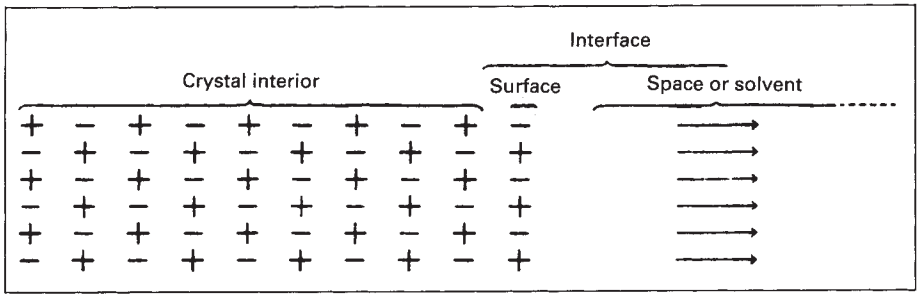

Fig. 4. *Schematic representation of the electric charge asymmetry at the crystal-solution interface (adapted from Ref. 52).*

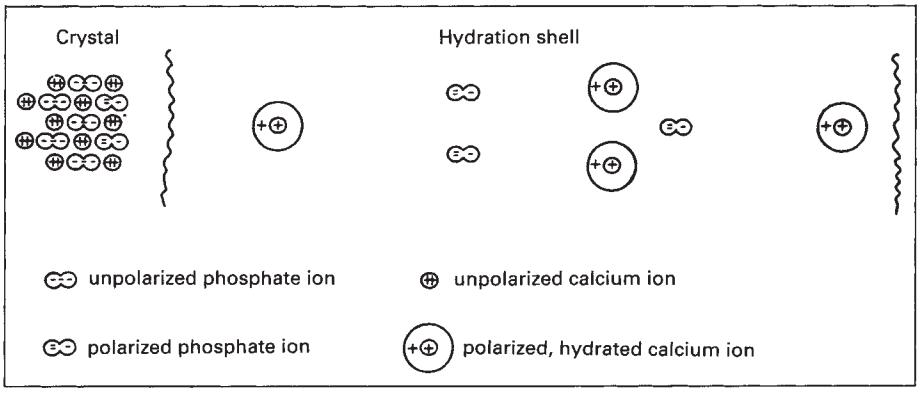

Fig. 5. *A schematic diagram of the hydration shell which was composed mainly of polarized hydrated calcium ions and polarized phosphate ions.* This arrangement decreased charge asymmetry at the hydroxyapatite crystal-water interface (adapted from Ref. 52).

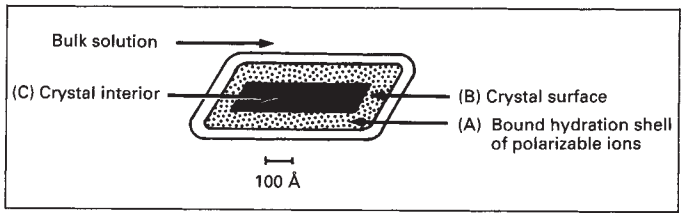

Fig. 6. *A diagramatic representation of a cross section view of hydroxyapatite crystal in aqueous suspension (adapted from Ref. 52).*

shell is fixed and static. When the crystals are in solution containing polarizable ions other than calcium and phosphate, there will be many substitutions and replacements in the hydration shell by ions which are normally foreign to the crystal lattice itself (discussed later).

Since it is generated by an electrostatic field, the hydration shell is tightly bound to the mineral crystal and will not be removed even by high-speed centrifugal force [52]. By virtue of its big surface area, the bone mineral will bind a large layer of hydration shell. On a volume basis, every crystal binds a hydration shell 1.9 times its own volume [52].

On the basis of the behavior of synthetic hydroxyapatite crystal in aqueous suspension a four compartment system can be delineated (Fig. 6): 1) the bulk solution; 2) the hydration shell; 3) the crystal surface; and 4) the crystal lattice interior. The bulk solution is the water compartment which provides the "raw materials" for the hydration shell (that is, an in vitro equivalent of the systemic circulation). This layer however, differs from the hydration shell in two respects: 1) It does not contain a high concentration of polarizable ions; and 2) it is easily removed by centrifugation.

Aside from its electroneutralizing properties, the hydration shell also serves as a bridge across which ionic interchange takes place between the bone mineral and its environment. In fact, bone can be pictured as a gigantic ion exchange column involving the transfer of ions to and fro across the hydrated crystal-solution interface. Some of the mineral constituent ions can be replaced by other ions of approximately the same radius, producing minor defects in the shape of the crystals which do not affect the overall structure [6]. Such substitutions can occur at the time the crystal is formed or by exchange with existing crystals. In vitro, interactions between bone mineral and solution involve diffusion into the hydration shell, exchange at the crystal surface and exchange within the crystals. The same processes probably occur in vivo. When there is a net transfer of ions from solution to the solid phase, crystal growth will result. Mineral dissolution is associated with net ionic transfer from solid to solution. Ion exchange, can, however, also take place when the solid and liquid are in equilibrium (zero net exchange). Theoretically, all ions in the extracellular fluid are able to enter the hydration shell of bone mineral, but the depth of further penetration is variable. The depth of penetration also determines the magnitude of ion exchangeability between bone and the extracellular fluid. The percentage of bone mineral which can undergo exchange of ions with the circulation can be determined by radioisotope measurements. Following injection of an isotope, specific activity is determined in bone and plasma and the ratio between the two yields the fraction of the exchangeable ion out of the total bone content of that ion. Based on such measurements three groups of ions can be recognized [39] (Table 3). As can be seen, potassium and chloride are confined to the hydration shell and are most rapidly and completely exchangeable. Magnesium, sodium and carbonate can penetrate the surface position in the crystal lattice and are less exchangeable. Finally calcium and phosphate can penetrate the crystal interior and are most slowly exchangeable. The ionic interchange between the crystal and its environment can be in one of two forms: Normal lattice ions—calcium and

Table 3. Participation of ions in hydroxyapatite-solution interaction

Ion	Penetration of ion into: Hydration shell	Crystal surface	Crystal interior	Ion displaced
K^+	+	−	−	−
Na^+	+	+	−	Ca^{++}
Mg^{++}	+	+	−	
Sr^{++}	+	+	+	
Ca^{++}	+	+	+	
Cl^-	+	−	−	
$Citrate^{\equiv}$	+	−?	−	$PO_4^{\equiv}$
$CO_3^{\equiv}$	+	+	−	
$PO_4^{\equiv}$	+	+	+	
F^-	+	+	+	OH^-

phosphate can exchange for their radioactive counterparts—isoionic exchange. Alternatively, the exchange can also be heteroionic namely, various cations displacing calcium and various anions displacing phosphate or hydroxyl group. The heteroionic exchange allows a wide variety of substitutions. It is more commonly found in the crystal surface and hydration shell layers than in the crystal interior where space-charge requirements are more restrictive.

In fact, this type of exchange permits the hydroxyapatite crystal to mirror the composition of fluids in which they are placed.

The concept of ionic interchange is crucial for full understanding of the mechanisms by which the skeleton participates in buffering acute and chronic metabolic acidosis. These mechanisms are specifically outlined in the following sections.

Buffering of acid outside the extracellular fluid

Acute acid loads have been shown to titrate extra and intracellular buffers [53, 54]. The state of titration of these buffers is reflected by the level of serum bicarbonate. With sustained acid loading, serum bicarbonate ultimately stabilizes at some reduced level despite continuing acid retention, indicating that an additional buffer system is titrated. In 1917, Van Slyke and Cullen [55] showed that only one-sixth of an acid load, infused into a dog, is neutralized by blood buffers, five-sixths presumably being neutralized by bicarbonate in interstitial fluid and lymph and by intracellular phosphate and proteinate buffers. Swan and Pitts [53] found that when 10 mM of hydrochloric acid per kilogram of body weight are infused into a nephrectomized dog only 43% of the infused acid is neutralized by bicarbonate present in extracellular fluid, whereas 57% is neutralized by a process in which hydrogen ions are exchanged for sodium and potassium ions which diffuse into extracellular fluid from cells. While these initial studies focused attention on intracellular buffers in general, it is now clear that both soft tissue, particularly skeletal muscle, and bone play a crucial role in buffering systemic pH changes. The weight of experimental evidence suggests that skeletal muscle is the critical repository of the hydrogen ions that depart from the extracellular compartment during the very early stages of acute metabolic acidosis [56–62]. Bone is involved in buffering after several hours following induction of acidosis [63–66] as well as during the chronic stages of metabolic acidosis [2, 3, 67–69].

Physico-chemical reactions involved in the buffering of acid by bone

Disturbances in the acid-base status of the organism induce prominent changes in the chemical composition of bone. These changes may differ between metabolic and respiratory changes, between acidosis and alkalosis, between organic and inorganic acidosis and most importantly, between acute and chronic stages of acid-base perturbations. Scanning of the literature reveals that while much emphasis has been put on the events occurring in acute and chronic metabolic acidosis, there is insufficient data regarding the role of bone in the buffering of alkali load or during respiratory changes.

Data accumulated both from in vivo and in vitro studies show that lowered extracellular pH cause buffering of protons by bone, and a loss of bone calcium, both during acute and chronic stages. The preparation of neonatal mouse calvariae has been extensively used to study the in vitro response of bone mineral to metabolic acidosis [70–73]. Using this model, Bushinsky showed that when the culture medium is acutely acidified by reducing the bicarbonate concentration (thus simulating a condition of metabolic acidosis), there is a net influx of protons into the neonatal mouse calvaria [70, 72]. Concomitant with proton influx there is a calcium efflux from the bone into the medium and the two fluxes are inversely correlated [72].

During acute respiratory acidosis, produced by elevating pCO_2 in the medium, there was no net proton flux and also there was less calcium efflux from bone during respiratory than during metabolic acidosis. The calcium efflux in respiratory acidosis was, in any case, significantly high compared to neutral pH conditions [74]. The smaller calcium efflux during respiratory acidosis is consistent with the clinical observation that respiratory acidosis does not appreciably increase urine calcium excretion [75, 76], and suggests that under this condition the bone plays less active role in buffering.

In metabolic acidosis, the influx of protons and efflux of calcium are not stoichiometrically linked. Thus, in acute metabolic acidosis, the buffering of protons entering the bone is not associated with major calcium release from the skeleton [72, 73]. Measurements of proton and calcium fluxes in neonatal mouse calvaria cultured for three hours in acidic medium, showed that between 16 and 21 nEq of protons entered the bone in exchange for each nEq of calcium that left [72]. Were all buffering linked to calcium release, one would expect a stoichiometric ratio of approximately 1:1 rather than 16 to 21:1. Since most of the bone calcium is trapped within the crystal lattice, this also means that short-term hydrogen ion buffering does not involve major mineral dissolution.

Several lines of evidence suggest that the chemical reactions taking place in acute metabolic acidosis involve significant changes in the bone content of CO_2, as well as of sodium and potassium. These changes are not necessarily associated with disruption of the crystal structure of the bone mineral since in these early phases the protons exchange with various chemical groups which reside on the surface of the hydroxyapatite crystal and as such are freely exchangeable (see **Bone composition**).

Bone CO_2

Bone is the main CO_2 reservoir in living organisms and accounts for approximately 5 moles of the 6 moles total CO_2

stores in the human adult [77]. For a long time it has been assumed that carbonate (CO_3^{2-}) was the main form of bone CO_2, and that it was deeply buried within the mineral phase and exchanging at a slow rate with the surrounding fluids [78]. In 1965, Pellegrino, Blitz and Rogers [79] demonstrated that bone carbonate is heterogenous and exists in two distinct forms, one of which is labile in long standing acidosis and not linked to the structure of the bone crystals. Later, Neuman and Mulryan [80] while studying the structure of synthetic apatite crystals, demonstrated that 40% of the bone CO_2 was rapidly exchangeable with the CO_2 of the surrounding milieu and was probably located on the crystal surface. This labile CO_2 appeared to be bicarbonate since half of it is lost on heating according to the formula $2HCO_3^- \rightarrow CO_2 + CO_3^{2-} + H_2O$. The remainder 60% of bone CO_2 is locked within the crystal lattice as carbonate (CO_3^{2-}) and is not at all, or poorly exchangeable. A further support to this concept of two CO_2 compartments in bone has been provided by the works of Payart et al [81, 82] who measured CO_2 in rat cortical bone with an accurate manometric technique and also determined the exchange of radioisotopically labelled bicarbonate between bone and the extracellular fluid.

The first demonstration of acute changes in CO_2 content of bone in metabolic acidosis was made by Irving and Chute in 1932 [83]. They found that bone lost substantial amounts of carbonate following four days of oral HCl administration to rats and guinea pigs. Burnell [68] has shown that metabolic acidosis of 5 to 10 days duration, induced by hydrochloric acid in adult mongrel dogs, leads to a decrease in bone carbonate by approximately 10% whereas metabolic alkalosis of the same duration induces an increase in bone bicarbonate by approximately 4%. No major changes in bone calcium and phosphorus concentrations were found in either condition. He theorized that bone CO_2 loss in metabolic acidosis takes place in two stages: 1) a rapid (hours) decrease in bone carbon dioxide without a release of calcium and phosphorus during the first hours of metabolic acidosis; and 2) long-term (days to weeks) mobilization of CO_3^{2-} from bone which also requires mobilization of Ca and P [3, 84, 85]. Bettice [86] has recently shown that the total carbon dioxide content of bone taken from rats decreases during metabolic acidosis and is directly proportional to the extracellular fluid bicarbonate concentration. The reduction in skeletal carbon dioxide occurred within the first 24 hours after the onset of metabolic acidosis and was found both in organic (diabetic ketoacidosis) and inorganic (HCl loading) types of acidosis. The role of carbonate in the buffering of protons has also been demonstrated in vitro by Bushinsky and Lechleider [87]. They showed that proton-induced calcium efflux from neonatal mouse calvariae cultured in acidic medium is due to dissolution of bone calcium carbonate.

In respiratory acidosis, it has been suggested that bone bicarbonate content is increased as a function of the ambient pCO_2 [81, 88]. The explanation is as follows: gaseous CO_2 hydrates with bone water to form carbonic acid which dissociates into one HCO_3^- and one H^+. The H^+ is taken up by the available carbonate ions to form a second HCO_3^-. Thus, upon CO_2 exposure, each bound CO_2 molecule will lead to the formation of two bicarbonate ions according to the following steps.

1) $CO_2 + H_2O \rightarrow H_2CO_3$

$H_2CO_3 \rightarrow H^+ HCO_3^-$

$H^+ + CO_3^{2-} \rightarrow HCO_3^-$

This concept is consistent with the in vitro finding that in respiratory acidosis, unlike metabolic acidosis, there is no net proton influx into the bone [74].

Role of bone sodium potassium and magnesium in buffering mechanisms

Aside from the large calcium pool contained in the skeleton, bone also contains abundant amounts of other cations, mainly sodium, potassium and magnesium [29, 39, 89]. The bone contains 35% of total body sodium for a total amount of 1400 mmol sodium in a 70 kg body. Approximately half of this amount is incorporated into the crystal lattice and is not readily exchangeable with the extracellular fluid whereas the rest, located on the crystal surface and in the hydration shell [39], is readily mobilized. The rapidly exchangeable fraction for sodium in vivo is about 45% in young animals and about 25% in the adult human [90]. Potassium which amounts to approximately 75 mmol in a 70 kg body does not enter the crystal [29], so that apart from a small amount in bone cells, potassium mainly resides in bone tissue fluid and in the hydration shell. Potassium is almost completely exchangeable in vitro but only about 60% exchangeable in vivo [91]. It is unknown what is the exact role of the excessive potassium accumulation in the bone extracellular fluid. Bone magnesium constitutes about 60% of the total body stores of this cation approximating a total amount of 800 mmols in an adult bone. Forty% of this amount is exchangeable with the surrounding environment.

The response of bone cations to challenges of either ion dietary deficit or metabolic acidosis seems to differ between sodium and potassium versus magnesium. Bone magnesium will be mobilized mainly during magnesium deficiency while systemic pH changes do not appear to change the magnesium stores significantly [3, 92, 93]. In contrast, bone sodium and potassium are mobilized more easily by metabolic acidosis than by depletion of the ion [89] and are thus considered to be a major line of defense, particularly during acute metabolic acidosis. A decrease in systemic pH is thought to cause the additional protons to displace sodium and potassium from the bone environment, resulting in an egress of sodium and potassium and a buffering of systemic acidity. Evidence for sodium hydrogen exchange is indirect and derives from both in vivo and in vitro studies. Dogs given an acid load buffer over half of the additional protons outside of the extracellular fluid [53]. The proton buffering is accompanied by a release of sodium into the extracellular fluid. In man, during the early days of metabolic acidosis, induced by ammonium chloride, negative sodium (−100 mEq) and potassium (−138 mEq) balances developed prior to the loss of body calcium [3]. Induction of metabolic acidosis by different methods in rats and dogs is followed by a decrease in the quantity of bone sodium [60, 63, 68, 94]. Bergstrom and Wallace [89] found that average normal rat bone contains 152 mEq of sodium and 26 mEq of potassium per kilogram of fresh bone. When rendered acidotic by intraperito-

neal dialysis against ammonium chloride, loss of bone sodium and potassium took place within 48 hours. Young rats lost 21 mEq of sodium and 7 mEq of potassium per kilogram of bone. In the adult group there was a loss of 54 mEq of sodium and 20 mEq of potassium per kilogram of bone. Also, by prelabeling bones of adult rats and dogs with ^{22}Na, Bettice and Gamble [57] were able to document significant reduction in the skeletal content of sodium within the first five hours of metabolic acidosis. The fraction of radiolabel lost was the same whether prelabeling was carried out over 18 hours or four weeks, suggesting that rapidly exchanging sodium was responding to the acid. Sodium was lost at different rates from different bones [57, 95]. Thus decreases in sodium content were greatest in the smaller bones of the rat, whereas in the dog the losses from flat bones exceeded those of the long bones [57]. Using the in vitro model of cultured neonatal mouse calvariae, Bushinsky, Levi-Setti and Coe measured bone Na,K and Ca by employing a high-resolution scanning ion microprobe [73]. They found that in control medium (pH = 7.4) the calvarial bone was rich in Na and K relative to Ca, whereas culture in a reduced pH medium (pH = 7.21) for three hours reduced the content of Na and K relative to Ca by approximately 90%.

The data presented so far indicate that during the acute phase of metabolic acidosis although there is calcium release from bone, there is little, if any, linkage between the extent of proton induced calcium release and the magnitude of proton buffering. Rather, proton buffering in the acute phase, involves mostly reduction in bone sodium, potassium and CO_2 content.

Calcium fluxes in chronic acidosis were studies in the in vitro model of cultured neonatal mouse calvariae [96]. Cells were cultured for 99 hours in control medium or in medium acidified (pH = 7.1) either by addition of HCl to the medium (metabolic acidosis) or by increasing the PCO_2 (respiratory acidosis). Over the first 48 hours there was greater net calcium efflux in metabolic than in respiratory acidosis. Both groups showed greater net calcium efflux than controls. However over the last 51 hours of the chronic 99 hour culture, there was net calcium efflux from the bone only during metabolic acidosis. Thus both in chronic and acute acidosis, decreased medium bicarbonate (metabolic acidosis) and not just a fall in pH is necessary to enhance net calcium efflux. This study did not provide a stoichiometric relationship between proton and calcium fluxes. However Lemann, Litzow and Lennon [3], carrying out balance studies in men, demonstrated an equivalence between proton retention and calcium excretion 12 days after the discontinuation of oral NH_4Cl, suggesting that unlike acute acidosis, the response to chronic acidosis involves increased bone dissolution and calcium egress into the the extracellular fluid. Interestingly, phosphorus losses accompanied calcium losses but these did not occur simultaneously. Thus phosphorus losses began immediately with acid loading and stopped abruptly when the load was discontinued. Phosphorus losses were ultimately restored, although significant calcium losses persisted even late in the recovery period. Analysis of bone composition in patients with long standing uremia revealed a significant decrease in calcium and carbonate which was proportionate to the duration of the disease [97]. Concentrations of phosphorus, sodium, potassium, magnesium and chloride were not altered. This study lends further support to the notion that skeletal sodium and potassium participate in the defense against metabolic acidosis only in the acute phase, whereas in the chronic stages calcium carbonate efflux constitute the main buffering mechanism.

Table 4. Ionic fluxes in metabolic and respiratory acidosis

	Metabolic acidosis		Respiratory acidosis	
	Acute	Chronic	Acute	Chronic
H^+ influx	↑ ↑ ↑	↑ ↑ ↑	—	—
Ca^{2+} efflux	↑ ↑	↑ ↑ ↑	↑	?
Efflux of ions other than Ca^{2+}	HCO_3^- Na^+ K^+	$CO_3^= \cdot PO_4^=$	—	—

Since carbonate and not phosphorus is the major anion accompanying calcium ion in chronic metabolic acidosis, its source could be either the hydroxyapatite crystal itself or alternatively, it could derive from a separate calcium carbonate crystal phase. Regardless of the source for calcium carbonate, it is clear that in chronic stages of metabolic acidosis, as opposed to the acute phase, significant reduction in the total mineral content of bone will take place along with dissolution of the bone crystal [3, 97].

Table 4 summarizes our current knowledge regarding the participation of various ions in the defense against systemic acidosis, (metabolic vs. respiratory).

Role of cell versus non-cell mediated mechanisms in the process of acid buffering by bone

Cultured medium acidified either acutely or chronically induces net calcium efflux from neonatal mouse calvariae [70–74, 96]. The in vitro studies are buttressed by in vivo data in humans and animals showing that bone salts are dissolved in response to metabolic acidosis or following the ingestion of ammonium chloride [3, 98–101]. Several studies have been carried out to assess the relative role of direct dissolution of bone mineral by protons as opposed to active osteoclastic (= cell mediated) resorption of bone in causing the net result of mineral loss. Dominguez and Raisz [102] studied rat fetal bones prelabeled with ^{45}Ca. They reported that over a wide pH range (6.9 to 7.5) H^+, CO_2 or HCO_3^- concentrations did not influence cell-mediated bone resorption. On the other hand, calcium release from devitalized bones (termed "non-cell-mediated calcium release") was linearly related to H^+ concentration. These authors concluded that their data do not support the suggestion that acidosis (acute and chronic) stimulates osteoclastic resorption directly. Bushinsky et al [71, 72, 87] found that during acute acidosis the cellular component of calcium flux is independent of medium pH: the effect of acute lowering of the pH is mediated through physiochemical factors alone and does not require participation of bone cells. This conclusion was based on the finding that both in acute metabolic and respiratory acidosis, calcium efflux from calvariae bone was not significantly different between live and dead (produced by 3 freeze-thaw cycles) cells. In contrast, in chronic metabolic acidosis only live but not dead neonatal mouse calvariae induced calcium efflux, thus establishing the role of bone cells in mineral dissolution during chronic as opposed to acute metabolic acidosis [96]. Goldhaber and Rabadjija [103] also demonstrated enhanced cell-mediated calcium release from

Table 5. Mean $[Ca^{2+}]_{in}$ and pH_i during osteoclast acidification

	Na^+-butyrate		$NaHCO_3$		$NaHCO_3$ removal, experimental
	Basal	Experimental	Basal	Experimental	
$[Ca^{2+}]_{in}$ *nM*	114 ± 18	56 ± 9[a]	145 ± 26	225 ± 37[b]	110
$\Delta[Ca^{2+}]_{in}$ *nM*		−58		80	97
pH_i	7.16 ± 0.1	6.56 ± 0.2[a]	7.05 ± 0.04	7.6 ± 0.05[b]	7.0 ± 0.1
ΔpH_i		−0.6		0.55	−0.48

Data are mean ± SE.
[a] $P < 0.01$
[b] $P < 0.03$
Osteoclasts were isolated from the medullary bone of laying hens fed a calcium deficient diet and then cultured on glass coverslips in MEM+ 10% FCS. Measurements of intracellular Ca^{2+} and pH_i were done by using the fluorescent dyes, Fura-2 and BCECF, respectively. Lowering of pH_i was achieved by two methods: 1) incubating the cells with 25 mM Na^+-butyrate; 2) incubating the cells with 25 mM $NaHCO_3$ followed by removal of HCO_3^-. In the latter condition cytosolic acidification was attributable to the operation of Cl^-/HCO_3^- exchanger since it was Cl^- dependent and DIDS inhibitable. (Reproduced with permission from Ref. 113).

neonatal mouse calvariae cultured for one week in acidic medium (metabolic acidosis). They found that both mineral and organic matrix, were removed simultaneously. Histologic examination of the extensively resorbed calvaria demonstrated the presence of numerous osteoclasts in different stages of bone destruction. The role of osteoclasts was also demonstrated by the fact that thryocalcitonin, an inhibitor of osteoclastic function abolished the proton-induced calcium release. It is not totally clear why different in vitro studies have come to different conclusions with regard to the role of bone cells in bone dissolution particularly under conditions of chronic metabolic acidosis [96, 103 vs. 102]. The disagreement between the various studies might be partially related to different cell preparations (neonatal mouse calvariae vs. mineralized shafts of radii and ulnae from fetal rats) or different experimental procedure (measuring net bidirectional calcium flux vs. unidirectional release of prelabeled ^{45}Ca).

In vivo studies are consistent with enhanced cell-mediated calcium efflux during chronic metabolic acidosis. Kraut, Mishler and Kurokawa [104] found that rats fed NH_4Cl for 16 hours developed a significant rise in serum calcium. This abnormality was independent of any PTH effect since the rats underwent TPTX at least 48 hours before the study. When cell-mediated calcium release from bone was inhibited with colchicine or calcitonin there was little change in serum calcium in response to the acidosis, suggesting an abolition of cell-mediated bone resorption. In another study Kraut et al [105] found that after two weeks of oral ammonium chloride, there was increased bone resorption in the epiphyseal but not in the cortical or metaphyseal bone in acidemic thyroparathyroidectomized rats compared with similar nonacidemic animals. Chan et al [106] found that in uremic rats, (5/6 nephrectomy), three months of oral hydrochloric acid led to an increase in the resorption surfaces with a significant diminution in trabecular bone areas compared with uremic rats not given acid. These bone changes were associated with high osteoclastic densities. That study, however, did not determine the effects of acidosis on bone in nonuremic rats. Interestingly, bone dissolution by osteoclasts from causes other than primary systemic acidosis, may in turn mobilize bicarbonate from the skeleton into the systemic circulation. Such is the case, for example, in chronic phosphate depletion which is a state associated with markedly enhanced bone resorption [107]. Under steady state conditions, phosphate depletion does not lead to abnormalities in acid-base homeostasis in spite of severe bone dissolution [108]. This phenomenon results from two coexisting and offsetting disturbances, both of which are related to phosphate depletion. These are: the bone resorption and impaired renal bicarbonate reabsorption leading to bicarbonaturia [109]. Indeed, abolishing bone resorption with colchicine, which blocks osteoclastic action, resulted in metabolic acidosis, and conversely when bicarbonaturia was eliminated by nephrectomy, metabolic alkalosis developed rapidly [110]. Thus, states associated with marked bone resorption are a potential cause for metabolic alkalosis.

Several theories have been proposed to explain the phenomenon of proton-mediated osteoclastic bone resorption. Lowered pH at the resorbing bone cell-mineral interphase might contribute to rapid removal of bone mineral and speed up the exposure of the organic matrix to various acid hydrolases elaborated by osteoclasts or to collagenases secreted by macrophages and/or other cells in the vicinity. This hypothesis would be in accord with the proposal of Baron et al [111], stating that osteoclasts are polarized cells that secrete protons and acid hydrolases into the bone resorbing compartment, thereby leading to the extracellular digestion of the mineral and organic phases of the bone matrix. An alternate hypothesis regards the effect of lowered pH on the activity of various transport pathways possessed by the osteoclasts. Acid extruding mechanisms, like the Na^+/H^+ antiporter or H^+ translocating ATPase, may be activated by the addition of protons to the environment. This in turn will lead to increased secondary hydrogen ion exit, giving rise to a higher local concentration of hydrogen ions at the cell-mineral interphase and thus provide the optimum milieu for the action of acid hydrolases secreted by the osteoclast. Recently, a Cl^-/HCO_3^- exchanger has been identified in the osteoclast [112]. A change in the activity of this exchanger, brought about by changes in medium pH or medium bicarbonate concentration, may affect osteoclastic function. Most interestingly, a recent finding showed that extracellular acidification of osteoclasts (metabolic acid) leads to a fall in both intracellular pH and cytosolic Ca^{2+} (Table 5). This in turn enhances formation of podosomes which are microfilament containing structures responsible for the adhesion of the osteoclast to the bone matrix (Fig. 7). Thus, acidification of the osteoclast environment may directly trigger the process of bone resorption [113]. Finally, the

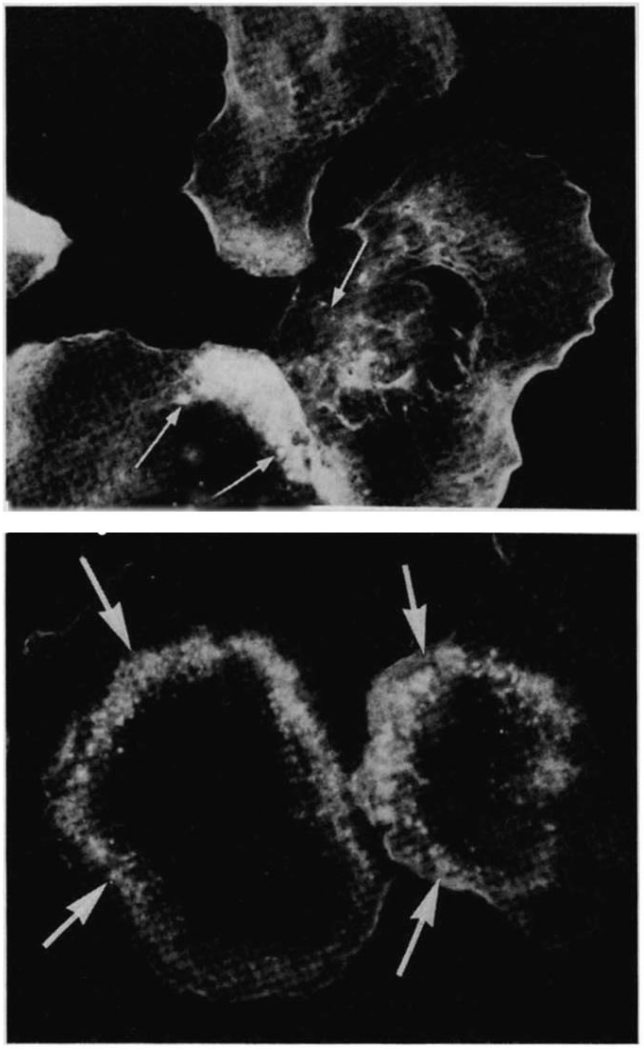

Fig. 7. *Induction of podosomes formation by metabolic acid.* These dot-like adhesion structures were rich in F-actin and were localized in an area within the osteoclast that binds it to bone. The expression of podosomes was detected by a fluorescent microscope, using rhodamine conjugated phalloidin which specifically binds F-actin. **Top panel.** Osteoclasts grown in control media pH 7.4 F-actin was distributed in a fine network with small number of podosomes observed (arrows). **Bottom panel.** Osteoclasts exposed to Na^+-butyrate for 90 min (pH = 7) showed well-organized multilayered podosomes. (Reproduced with permission from Ref. 113).

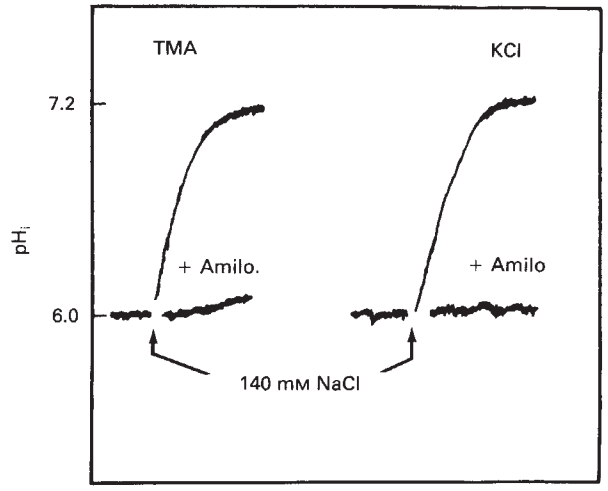

Fig. 8. *Na^+/H^+ exchange in osteoblasts.* UMR-106 cells (an osteoblast phenotype cell line) that were depleted of Na^+ and acidified to intracellular pH (pH_i) of 6.0 were loaded with the pH fluorescent dye, BCECF. When added to Na^+-free solutions (TMA, KCl) no change in fluorescence was observed. However, adding the cells to a media containing 140 mM Na^+ resulted in recovery from the acid load which was completed when the cells attained their original resting pH_i of approximately 7.2. The acid recovery process was inhibited by amiloride. Thus osteoblasts recovered from an acid pulse by a Na^+-dependent amiloride-sensitive mechanism, indicating that they possessed Na^+/H^+ exchanger (reprinted from Ref. 117 with permission).

coupling between osteoclast and osteoblast activities may be important for acid-induced osteoclastic bone resorption. Several recent studies indicate that the osteoblast may play a pivotal role in the bone resorption process which is ultimately carried out by the osteoclast [114]. The osteoblast serves as a target cell for PTH and other bone resorbing hormones and as a potent producer of prostaglandins and various cytokines with bone resorbing activity [9–14, 115, 116]. Based on these studies, it is conceivable that hydrogen ion acts on osteoblasts to produce agents that in turn stimulate osteoclasts. Also, we have shown that the osteoblast possesses two pH regulatory mechanisms: Na^+/H^+ exchanger (Fig. 8) and Cl^-/HCO_3^- exchanger (Fig. 9) [117, 118]. Thus changes in the pH of the environment which will alter the intracellular pH might affect the activity of either one of these exchangers. This in turn may stimulate osteoclastic activity by some "coupling" or paracrine mechanisms.

If indeed osteoblasts play a role in proton mediated bone resorption, it is by no means the only operative mechanism. This idea is based on the work by Arnett and Dempster [119] which provides a direct evidence that hydrogen ion does indeed directly stimulate the activity of osteoclasts and does not need to be mediated by any other cell types. They showed that neonatal rat osteoclasts placed on thin slices of dead cortical bone gave rise to a significant increase in the number and surface area of "resorption pits" when the pH of the incubation media was lowered. PTH had no additional resorptive activity while calcitonin produced near total inhibition.

The data on cell versus non-cell mechanisms for bone resorption in respiratory acidosis are not as clear as with metabolic acidosis. In the in vitro model of neonatal mouse calvariae acute respiratory acidosis induces non-cell-mediated calcium release from bone [96]. In chronic in vitro respiratory acidosis calcium efflux was not observed at all regardless of whether the cells are dead or alive [96]. However, studies using measurements of unidirectional release of prelabeled ^{45}Ca have shown increased calcium release from bone through non-cell mediated processes in chronic respiratory acidosis as well as in metabolic acidosis [102]. There is a paucity of data from experiments studying the effect of respiratory acidosis on bone in vivo. Measurements of divalent ion excretion in the urine (as a marker for bone resorption) show that neither acute nor chronic respiratory acidosis appreciably increase urinary calcium [75, 76, 120–125]. However, in chronic respiratory acidosis there appears to be an increase in total serum calcium [120, 126],

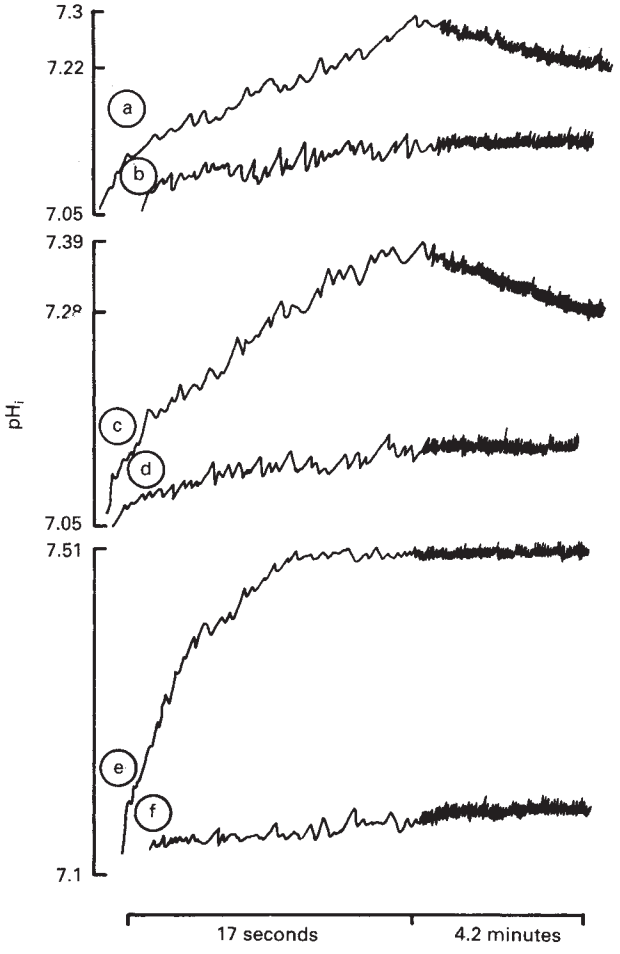

Fig. 9. *$Cl^-/HCO_3^-/OH^-$ exchanger in osteoblasts.* UMR-106 cells (an osteoblast phenotype cell line) were loaded with the pH fluorescent dye, BCECF and suspended in Cl-free media (Na^+ gluconate or K^+ gluconate) **(a,c,e)**. An immediate alkalinization was observed which resulted from efflux of intracellular Cl^- in exchange for extracellular base equivalents. The base equivalents included either OH^- ions as demonstrated in **(a)**, where the solution was bubbled with 100% O_2 to eliminate any CO_2/HCO_3, or HCO_3^- itself, as demonstrated in **(c)** (solution equilibrated with air) and **(e)** (addition of 4 mM HCO_3^- to the solution). In each condition the alkalinization was blocked by the stilbene derivative DIDS, which is a known blocker of Cl^-/HCO_3^- exchange (b,d,f) (reprinted with permission from Ref. 118).

suggesting either increased bone mineral dissolution or increased intestinal calcium absorption (or both) coupled to increased renal tubular calcium reabsorption.

Role of PTH

The data obtained from the in vitro experiments as well as in vivo studies using thyroparathyroidectomized animals, indicate that low extracellular pH (mainly metabolic acidosis) directly induces bone dissolution regardless of the presence of absence of PTH. One needs to realize, however, that in the intact organism, systemic acidemia may induce either a change in the synthesis or secretion rate of PTH or alter the activity of the hormone as a bone resorbing agent [127–131] or do any of these in combination. Thus PTH may play a modulatory role on the bone resorbing effect of acidemia and conversely may augment buffering of acid by bone.

Studies in whole animals yielded conflicting results regarding the role of PTH. In one study, nephrectomized rats and dogs with intact parathyroid glands were compared to thyroparathyroidectomized (TPTX) animals with or without replacement of PTH. TPTX animals not given PTH showed 100% mortality following an acute acid load and seemed to buffer a greater proportion of the infused acid with extracellular fluid bicarbonate, as they became more acidemic than PTH-replete animals [132]. Pharmacologic doses of PTH infused into the TPTX animals prevented the otherwise universal mortality following HCl infusion, the animals appearing to buffer the administered hydrogen ion load in a manner similar to that of the non-TPTX animals. A second study [133] showed that nephrectomized and acutely TPTX rats buffered an acute acid load as well as nephrectomized rats with intact parathyroid glands although they had a higher mortality rate. However, when the acutely TPTX animals were given exogenous PTH they buffered the acid load to a greater extent than either TPTX non-treated rats or non-TPTX animals. Moreover, administration of EDTA or colchicine, agents that presumably stimulate endogenous PTH release, led to an increase in the buffering capacity of non-TPTX but not of TPTX animals. Also, secondary hyperparathyroidism, induced experimentally by both acute and chronic renal failure, enhanced buffering capacity of an acid load which was dependent on the presence of intact parathyroid glands. These observations suggest that high levels of PTH, whether exogenous or endogenous, may enhance the extrarenal buffering of an acute acid load. When TPTX animals were given acetazolamide prior to the administration of exogenous PTH, the hormone failed to enhance the buffering of the acid load, thus indicating that its effect is mediated through carbonic anhydrase. In contrast to these two studies, a third in vivo study [134] showed that nephrectomized TPTX rats buffered and survived an acute acid load the same as nephrectomized sham TPTX rats. In this study, stringent measures were taken to ensure comparable treatment and hemodynamic stability in all the animal groups studied. Maximum attention was paid to minimize surgical stress and to prevent hypocalcemia and hypothyroidism prior to the experiments. Also, the non-TPTX animals underwent an appropriate sham procedure in order to equalize the surgical stresses between the groups. These changes in the experimental design could partially account for the disparity between the third study [134] and the other two [132, 133].

In vitro experiments are also conflicting in their results. Using the model of neonatal mouse calvariae, Bushinsky [70] demonstrated lack of an effect of PTH (10^{-8} M) on proton fluxes during acute and chronic changes in media pH. In fact, PTH was found even to inhibit proton influx in acidic medium. Martin et al [128] examined the effects of acute acidosis in vitro on the uptake and action of PTH in an isolated perfused canine tibia preparation. Lowering perfusate pH to 7.0 to 7.1 (by HCl), as compared to control pH (7.4) resulted in increased arteriovenous difference for iPTH across the perfused bone. Moreover, both baseline and PTH stimulated cAMP generation increased significantly under the acidic conditions. These changes in the uptake of the hormone and its biological activity were observed only in metabolic acidosis since acidosis induced

by increasing perfusate pCO_2 (simulated respiratory acidosis) did not result in similar changes.

The relevance of the foregoing animal studies to the clinical state of acidosis in man is not clear. Acute metabolic acidosis has been found to reduce [127, 135] not to affect [136] or only slightly elevate serum PTH [129, 131]. Furthermore, even during chronic metabolic acidosis, in which bone is known to be the major source of buffer responsible for the stabilization of extracellular bicarbonate concentration, evidence that PTH is the critical mediator of this process is lacking. In this regard, Lemann, Litzow and Lennon [69] induced chronic ammonium chloride acidosis in hypoparathyroid individuals maintained on calcium and vitamin D supplementation and compared their response to that of normal volunteers fed equivalent amounts of acid. Their data indicated that the two groups were not different with respect to decrement in plasma bicarbonate concentration, positive hydrogen ion balance and degree of hypercalciuria observed. In another study, Coe et al [131] found that there was no temporal relationship between severity or onset of acidemia and elevation in the plasma level of PTH observed during chronic ammonium chloride acidosis in normal volunteers. In fact, the elevated PTH appeared to be a direct consequence of the attendant hypercalciuria and not of the acidemic state per se. Moreover, studies where positive hydrogen ion balance was produced in normal subjects by prolonged administration of ammonium chloride or feeding a high protein diet were unable to show elevations in plasma PTH, urinary cAMP or plasma levels of vitamin D metabolites. There was, however, significant hypercalciuria during the chronic metabolic acidosis which indicated that bone participated in the buffering process [135, 136].

From the currently available data, it can be concluded that PTH does not seem to play a critically important role in buffering an acute mineral acid acidosis. Likewise, there is not conclusive evidence that the skeletal buffering occurring during chronic metabolic acidosis is mediated by PTH.

Bone disease related to acidosis

The first experimental evidence for acidosis induced bone loss derives from the classical study by Jaffe, Bodansky and Chandler in 1932 [137]. In that study, an attempt was made to evaluate the independent effects of each of three factors: age, dietary calcium and chronic acid ingestion on the generation of bone decalcification. Studying dogs with ages ranging from 3 to 18 months old who were fed ammonium chloride for about three months, they found that the effect of acid on bone was strikingly dependent upon the calcium intake. Thus dogs receiving an adequate calcium diet and ammonium chloride showed less decalcification than those receiving the acid load while on low calcium diet. In the younger groups, ammonium chloride and low calcium diet were synergistic with respect to the causation of bone thinning whereas in the older animals the acid load did not have a marked additional effect to that of low Ca diet alone. On pathologic examination there was generalized thinning of both cortical and trabecular bone—osteoporosis. In the youngest age group on low calcium diet, together with ammonium chloride there was also considerable subperiosteal and subendosteal resorption, osteoclast increase and marrow fibrosis—osteitis fibrosa. Histologic evidence of rickets or osteomalacia was not observed in any group. The experimental evidence for the direct bone decalcifyinig effect of chronic acidosis was subsequently confirmed by other investigators [67, 138–141], though not all workers found acid induced losses in cortical bone [105, 106, 139, 140]. Some studies suggest that cortical and trabecular bone respond differently to the same stimuli [106, 142, 143]. Thus rats with mild to moderate uremia lose cortical bone, but in spite of an increase in osteoclast densities and resorption surfaces, trabecular bone areas remain normal. On the other hand, chronic mineral acidosis induced in uremic animals leads to further increases in osteoclasts and resorption surfaces with a significant diminution in trabecular bone areas but no further cortical bone loss. If, indeed, uremia diminishes cortical bone and acidosis diminishes trabecular bone, it may reflect different mechanisms for the bone loss induced by uremia, per se, as opposed to the acidosis associated with it. Thus, resorption of cortical bone in uremia could be related to secondary hyperparathyroidism which constitutes part of the uremic syndrome.

There are two main clinical entities of chronic metabolic acidosis in man. These are renal tubular acidosis and chronic uremic acidosis. Albright et al [144] made the distinction between acidosis related to "tubular insufficiency and impaired urinary acidification" where osteomalacia develops and "glomerular insufficiency", which signifies advanced renal failure. They defined the bone changes in the latter condition as "renal rickets" or "renal osteitis fibrosa". Osteomalacia resulting from tubular insufficiency without glomerular insufficiency responded remarkably to combined treatment with vitamin D and an alkalinizing salt. Once cured, only the alkalinizing salt was necessary to prevent further bone disease. Subsequently, renal tubular acidosis (RTA) has been shown to be a major cause of impaired growth and short stature, particularly evident in children [145–148]. Brenner et al [149] studied 56 children and 36 adults with RTA. He found that 67% of the patients with proximal RTA (type II) developed radiographic skeletal abnormalities which consisted of rickets (widened and irregular epiphysealmetaphyseal junction or evidence of bowing deformities) in all of the children and osteopenia in many of the adults. Children with RTA can achieve an immediate increase in their height velocity and even reverse stunted growth when treated with alkali in large amounts and as early in life as possible [148, 150–158]. Likewise, the bone disease of both children and adults with RTA can be healed by treatment with alkali alone and healing process can begin almost immediately after initiation of treatment [145, 154, 159–161].

It is not clear whether the pathophysiology of the bone disease associated with RTA, tightly resembles the bone damage observed in experimental chronic acidosis. Osteomalacia is usually not seen in experimental acidosis [105, 106, 137] while it is very commonly seen in children and adults with RTA [148, 149]. This disparity may be partially related to the fact that the skeletal abnormalities in RTA are multifactorial, and could be ascribed in addition to the acidosis per se, also to negative Ca^{2+} balance due to hypercalciuria, secondary hyperparathyroidism, hypophosphatemia and alteration in Vitamin D metabolism. Hypophosphatemia is often present in non-uremic patients with chronic metabolic acidosis and osteomalacia secondary to RTA [145–147] and the resolution of skeletal lesions of osteomalacia during alkali therapy is associated with marked improvement in phosphorus balance [145, 146]. Thus alterations in the metabo-

lism of phosphorus during chronic non uremic acidosis may be an important determinant in the development of osteomalacia in humans with RTA induced metabolic acidosis.

Altered vitamin D metabolism during acidosis

The literature relating to the effects of acidosis on the in vitro synthesis, the vivo production and plasma levels of $1,25(OH)_2D_3$ is confusing, largely because of the different experimental approaches employed. There is a great deal of evidence indicating that metabolic acidosis suppresses the production of $1,25(OH)_2D_3$ in vitamin D-deficient chicks [162–164] and rats [165–168] both in vitro [162, 166–168] and in vivo [163–165, 169]. However, some of these experimental systems resulted in alterations of a variety of factors known to affect 1α-hydroxylase activity. Thus, the acidotic chicks used by Sauveur et al [163] were hyperphosphatemic and the experimental conditions used by Baran et al [164] resulted in a rise in serum calcium. Obviously, both conditions could have independent effects on the production of $1,25(OH)_2D_3$. Moreover, Cunningham, Bickle and Avioli [168] reported that in vitamin D-deficient rats, the suppressive effect of acidosis on 1α-hydroxylase activity was much attenuated by day 6 and was no longer significant by day 21. Of considerable importance is the fact that most studies showing reduced $1,25(OH)_2D_3$ synthesis in acidosis used vitamin D deficient animals. In vitamin D-replete chronically acidotic animals or humans, vitamin D synthesis does not seem to be altered [170]. Gafter et al [170] found elevated plasma $1,25(OH)_2D_3$ concentrations in acidotic vitamin D replete rats and showed that the increments in plasma level of $1,25(OH)_2D_3$ in response to a low calcium and phosphate diet were normal in these animals. Similarly, Chan et al [106] found only a nonsignificant fall in serum $1,25(OH)_2D_3$ concentrations in acidotic uremic rats compared to non-acidotic uremic animals. Blood levels of vitamin D metabolites in human subjects were not altered by experimentally induced acidosis in the studies of Adams, Gray and Lemann [135] or Kraut et al [171], or in patients with congenital renal acidification defects reported by Chesney et al [172].

From the foregoing data, it seems unlikely that the occurrence of osteomalacia in humans with metabolic acidosis is due to alterations in the production or metabolism of 1,25 dihydroxyvitamin D. The different bone pathology in human RTA compared to experimental chronic acidosis should therefore be ascribed to other factors, as stated. These factors play a role together with the acidosis to bring about osteomalaic changes in RTA as opposed to the osteopenia of isolated experimental acidosis.

Uremic bone disease

The association of albuminuria and late rickets was noted as early as 1883 by Lucas [173] but it was not until 1911 that Fletcher [174] clearly recognized the etiologic relationship between chronic renal disease and bone deformities. Barber [175] reported 10 additional cases under the term renal dwarfism. Parsons [176] and Teall [177] gave the first description of the roentgenologic picture of renal rickets. The reports by Langmead and Orr [178], Smyth and Goldman [179], Shelling and Remsen [180], Albright et al [153] and others [181–183] served to emphasize the frequent occurence of diffuse parathyroid hyperplasia in renal osteodystrophy. The commonest histological abnormalities in uremic bone disease consist of osteomalacia, osteitis fibrosa, or aplastic changes in varying proportions. The etiology of uremic osteodystrophy is considered to be multifactorial, being related mainly to alterations in vitamin D metabolism, phosphate retention secondary hyperparathyroidism and aluminum deposition in bone [184]. It is therefore hard to evaluate the contribution of chronic uremic acidosis, per se, to the bone pathology. Balance studies [101] as well as direct analysis of bone composition from patients with chronic renal failure [97] clearly established the fact that in renal disease bone participates in the buffering of hydrogen ions which accumulate during uremic acidosis. The clinical significance of this phenomenon is not clearly defined. Ginzler and Jaffe [185], Albright and Reifenstein [186], Snapper [187] and Bartter [188] considered that the uremic osteitis fibrosa is mainly due to the acidosis of chronic renal failure. Moreover, in a group of 41 children with chronic renal disease, West and Smith [189] found acidosis in 76% of 21 stunted patients and in none of those growing normally. It must be realized, however, that even if uremic acidosis plays some role in the pathogenesis of the bone disease, the effect of administered alkali on bone pathology and resumption of normal growth is not as gratifying as in renal tubular acidosis [99, 190–194]. Furthermore, the effects of alkali on total body metabolism of calcium and phosphorus, in uremic patients, have also yielded conflicting results. Uremic acidosis differs from other forms of chronic experimental or clinical acidosis in that negative calcium balance, when present, is due to decreased net absorption from the gut rather than excessive renal loss [192–194]. The failure of calcium absorption was ascribed by some investigators to increased phosphorus excretion into the intestine which, in turn, forms insoluble calcium phosphate complexes thus preventing calcium absorption [187, 193, 195]. This theory has been criticized on the ground that in chronic renal failure, bone lesions may occur without hyperphosphatemia [192] and attempts to increase calcium absorption by binding phosphorus in the gut with aluminum hydroxide have usually failed. It was suggested, therefore, that calcium malabsorption is primary and not secondary to increased intestinal phosphorus content. An improved calcium balance by alkali therapy in patients with chronic renal disease has been demonstrated by Liu and Chu [193] and by Litzow, Lemann and Lennon [99]. However, Stanbury and Lumb [192], and Fletcher, Jones and Morgan [190] failed to demonstrate any effect of alkali either on mineral malabsorption or on uremic bone disease. Both abnormalities could however be corrected by large doses of calciferol [190, 192, 194].

It is apparent that, at the present, there is not enough data to suggest an etiologic association between uremic acidosis and uremic osteodystrophy. Admittedly, there are several case reports linking human uremic osteomalacia with acidosis [196–200]. However, in some of these studies tubular acidification defects were present in addition to renal failure while others failed to provide histological confirmation of improvement of bone pathology by alkali. Thus the currently available evidence purporting to show that acidosis is a major factor in the pathogenesis of uremic osteodystrophy is inadequate.

Summary and unresolved issues

In this review we presented the current information on the role of bone in the defense against acute and chronic acid loads. It is apparent that the same process that is involved in buffering of acid by bone will ultimately lead to reduction in bone mass and therefore the relationship between systemic acidemia and bone can be described as a "trade-off" phenomenon. Thus far the major bulk of both the in vivo and in vitro studies about bone and pH changes have concentrated on the effects of acute and chronic metabolic acidosis. Metabolic acidosis was induced mainly by HCl or NH_4Cl mineral acidosis. It seems that more studies need to be done in order to address the following issues.

1) The effect of chronic respiratory acidosis should be studied by using balance studies and comparing acid retention to mineral loss from bone, measuring bone composition ($Na^+ \cdot K^+ \cdot$ calcium and carbonate) and doing histologic and histomorphometric studies.

2) The effect of acute and chronic metabolic alkaloses on bone composition and bone histology have a limited number of data from in vitro studies available, but more chronic in vivo studies should be done to provide more information in this regard.

3) How does organic metabolic acidosis compare to inorganic (mineral) acidosis with respect to its effect on bone? The ccurrently available data deals mainly with mineral acidosis. More acute in vitro studies should be done in which either lactic acid or ketoacids are added to cultured media to lower its pH, and then proton and calcium fluxes between bone cells and media can be studied. Likewise, both experimental and clinical studies can be conducted to evaluate the effect of chronic organic acidosis. For the clinical part, patients with chronic lactic acidosis (D-lactic acid or L-lactic acid) can serve as study groups. The two main chronic metabolic acidoses in humans—RTA and zotemic acidosis—are basically mineral acidosis types and therefore more information about the effect of chronic organic acidosis on human bone is necessary. Also, uremic acidosis differs from RTA inasmuch the latter is equivalent to HCl acidosis while in chronic renal failure acidosis is related to the accumulation of sulfuric and phosphoric acid. While both are considered to be mineral acids they might theoretically have different effects on bone, especially the latter. Under the in vivo conditions high phosphate may have an independent "anabolic" effect on bone, while at the same time it may contribute to bone disease by lowering ionized calcium, thus leading to secondary hyperparathyroidism. Therefore to study the effect of the "uremic acids", in isolation, one has to carry out either an in vitro study where media of cultured bone is acidified by adding these acids or in vivo studies in TPTX animals loaded either acutely or chronically with phosphoric and sulphuric acid.

4) More information is necessary regarding the effect of age on acid-induced bone disease. Jaffe, Bodansky and Chandler [137] have elegantly demonstrated that in younger animals the skeletal pathology related to acid load is much greater than in adult animals. Whether this has any clinical implication is still unknown. Of particular interest is the question of whether age related or postmenapausal osteoporisis have synergistic or additive effect to that of acid-induced bone loss.

5) While much emphasis has been put on the role of the osteoclast in mediating acid-induced bone resorption, there has not been much attention drawn to the role of the osteoblast under these circumstances. This information is of utmost importance in view of the currently available data about the coupling between osteoclast and osteoblast activities. Enough evidence has now been accumulated which indicates that osteoblasts play a pivotal role in the bone resorption process which is ultimately carried out by the osteoclast. It is therefore important to study the in vitro effect of acute and chronic pH changes on osteoblastic function and on its response to hormones and growth factors.

6) The buffering of acid by bone has been mainly linked to dissolution of calcium carbonate either by a direct action of the acid or by the action of the osteoclast. It has recently been shown [118] that osteoblasts possess a Cl^-/HCO_3^- exchanger which under the physiologically existing ionic gradients, extrudes base from the cytosol into the bone environment. It is thus possible that this exchanger might aid in the buffering of acid penetrating the bone. Since Cl^-/HCO_3^- exchanger in osteoblasts is regulated by intracellular calcium [118] as well as by cAMP [201], it would be interesting to see if the osteoblast responds differently to these second messengers during acidosis compared to neutral pH and whether this leads to altered function of the exchanger.

It appears that more investigations are needed in order to evaluate the role of osteoblast- osteoclast interaction in the pathogenesis of acid-induced bone disease.

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References

1. Lemann J, Lennon EJ: Role of diet, gastrointestinal tract and bone in acid-base homeostasis. *Kidney Int* 1:275–279, 1972
2. Barzel US: The effect of excessive acid feeding on bone. *Calcif Tissue Res* 4:94–100, 1969
3. Lemann J, Litzow JR, Lennon EJ: The effects of chronic acid loads in normal man: Further evidence for the participation of bone mineral in the defense against chronic metabolic acidosis. *J Clin Invest* 45:1608–1614, 1966
4. Robey PG, Termine JD: Human bone cells in vitro. *Calcif Tissue Int* 37:453–460, 1985
5. Robey GH, Shen LWF, Young MF, Termine JD: The biochemistry of bone, in *Osteoporosis: Etiology, Diagnosis and Management*, edited by BL Riggs, LJ Melton, New York, Raven Press, 1988, pp. 95–109
6. Neuman WF, Neuman MW: *The Chemical Dynamics of Bone Mineral*. Chicago, The University of Chicago Press, 1958, pp. 39–54
7. Posner AS: Bone mineral and the mineralization process, in *Bone and Mineral Research 5*, edited by Peck WA, Amsterdam, Elsevier Science Publishers, B.V., 1987, pp. 65–99

8. ARMSTRONG WD, SINGER L: Composition and constitution of the mineral phase of bone. *Clin Orth* 38:179–190, 1965
9. MCSHEEHY PMJ, CHAMBERS TJ: Osteoblastic cells mediate osteoclastic responsiveness to parathyroid hormone. *Endocrinology* 118:824–828, 1986
10. MARTIN TJ, PARTRIDGE NC: Prostaglandins and cellular bone resorption, in *Prostaglandins and Cancer: First International Conference*, edited by POWLES TJ, BOCKMAN RS, HONN KV, RAMWELL P, New York, Liss, 1982, pp. 525–540
11. RODAN GA, MARTIN TJ: Role of osteoblasts in hormonal control of bone resorption—a hypothesis. *Calcif Tiss Int* 33:349–351, 1981
12. MCSHEEHY PMJ, CHAMBERS TJ: 1,25-Dihydroxyvitamin D_3 stimulates rat osteoblastic cells to release a soluble factor that increases osteoclastic bone resorption. *J Clin Invest* 80:425–429, 1987
13. RAISZ LG: Local and systemic factors in the pathogenesis of osteoporosis. *N Engl J Med* 318:818–828, 1988
14. CANALIS E, MCCARTHY T, CENTRELLA M: Growth factors and the regulation of bone remodeling. *J Clin Invest* 81:277–281, 1988
15. OWEN M: The origin of bone cells in the post natal organism. *Arthr Rheum* 23:1073–1080, 1980
16. IBBOTSON KJ, ROODMAN GD, MCMANUS LM, MUNDY GR: Identification and characterization of osteoblast-like cells and their progenitors in cultures of feline marrow mononuclear cells. *J Cell Biol* 99:471–480, 1984
17. ROODMAN GD, IBBOTSON KJ, MACDONALD BR, KUEHL TJ, MUNDY GR: $1,25(OH)_3$ vitamin D_3 causes formation of multinucleated cells with several osteoclast characteristics in cultures of primate marrow. *Proc Natl Acad Sci USA* 83:8213–8217, 1987
18. BARON R, VIGNERY A, HOROWITZ M: Lymphocytes, macrophages and the regulation of bone remodeling, in *Bone and Mineral Research, Annual 2*, edited by PECK WA, Amsterdam, Elsevier, 1984, pp. 175–243
19. PARFITT AM, KLEEREKOPER M: The divalent ion homeostatic system-physiology and metabolism of calcium, phosphorus, magnesium and bone, in *Clinical Disorders of Fluid and Electrolyte Metabolism*, edited by MAXWELL MH, KLEEMAN CR, New York, McGraw Hill, 1980, pp. 269–398
20. PARFITT AM: Bone and plasma calcium homeostasis. *Bone* 8 (supp. 1):S1–S8, 1987
21. PARFITT AM: Integration of skeletal and mineral homeostasis, in *Osteoporosis: Recent Advances in Pathogenesis and Treatment*, edited by DELUCA HF, FROST H, JEE W, JOHNSTON C, PARFITT AM, Baltimore, University Park Press, 1981, pp. 115–126
22. NEUMAN WF, RAMP WK: The concept of a bone membrane: Some implications, in *Cellular Mechanisms for Calcium Transfer and Homeostasis*, edited by NICHOLS G JR, WASSERMAN RH, New York, Academic Press, 1971, pp. 197–206
23. TALMAGE RV: Morphological and physiological considerations in a new concept of calcium transport in bone. *Am J Anat* 129:467–476, 1970
24. PARFITT AM: The actions of parathyroid hormone on bone: Relation to bone remodeling and turnover, calcium homeostasis and metabolic bone disease. I. Mechanisms of calcium transfer between blood and bone and their cellular basis: Morphologic and kinetic approaches to bone turnover. *Metabolism* 25:809–844, 1976
25. TALMAGE RV, GRUBB SA: A laboratory model demonstrating osteocyteostoblast control of plasma calcium concentrations. *Clin Orthoop* 122:299–306, 1977
26. ROBIONSON RA: Chemical analysis and electron microscopy of bone, in *Bone as a Tissue*, edited by RODAHL K, NICHOLSON J, BROWN EM, New York, McGraw-Hill, 1960, pp. 186–250
27. TALMAGE RV, COOPER CW, TOVERUD SU: The physiological significance of calcitonin, in *Bone and Mineral Research Annual 1*, edited by PECK WA, Amsterdam, Excerpta Medica, 1983, pp. 74–143
28. HOWARD JE: Present knowledge of parathyroid function with special emphasis on its limitations, in *Ciba Foundation Symposium on Bone Structure and Metabolism*, edited by WOLSTENHOLME GEW, O'CONNOR CM, Boston, Little Brown, 1956, p. 206
29. CANAS F, TEREPKA AR, NEUMAN WF: Potassium and the milieu interieur of bone. *Am J Physiol* 217:117–120, 1969
30. DAVIS WL, MATTHEWS JL, MARTIN JH, KENNEDY JW, TALMAGE RV: The endosteum as a functional membrane, in *Calcium Regulating Hormones*, edited by TALMAGE RV, OWEN M, PARSONS JA, Amsterdam, Excerpta Medica, 1975, pp. 275–283
31. GEISLER JZ, NEUMAN WF: The membrane control of bone potassium. *Proc Soc Exp Biol Med* 130:608–612, 1969
32. PARADIS GR, BASSINGTHWAIGHTE JB, KELLY PJ: Inhibition of transport of ^{47}Ca and ^{85}Sr by lanthanum in canine cortical bone. *J Appl Physiol* 36:221–225, 1974
33. SCARPACE PJ, NEUMAN WF: Quantitation of calcium fluxes in chick calvaria. *Biochim Biophys Acta* 323:267–275, 1973
34. SCARPACE PJ, NEUMAN WF: The blood:bone disequilibrium I. The active accumulation of K^+ into the bone extracellular fluid. *Calcif Tissue Res* 20:137–149, 1976
35. SCARPACE PJ, NEUMAN WF: The blood:bone disequilibrium II. Evidence against the active accumulation of calcium or phosphate into the bone extracellular fluid. *Calcif Tissue Res* 20:151–158, 1976
36. TALMAGE RV: Calcium homeostasis- calcium transport-parathyroid action. *Clin Orthopaedics Related Res* 67:210–224, 1969
37. NEUMAN WF, NEUMAN MW, BROMMAGE R: Aerobic glycolysis in bone: Lactate productoin and gradients in calvaria. *Am J Physiol* 234:C41–C50, 1978
38. BUSHINSKY DA, CHABALA JM, LEVI-SETTI R: Ion microprobe analysis of mouse calvariae in vitro: Evidence for a "bone membrane". *Am J Physiol* 256:E152–E158, 1989
39. TRIFFITT JT, TEREPKA AR, NEUMAN WF: A comparative study of the exchange in vivo of major constitutents of bone mineral. *Calcif Tissue Res* 2:165–176, 1968
40. NEUMAN WF: The milieu interieur of bone: Claude Bernard revisited. *Fed Proc* 28:1846–1850, 1969
41. LOEWENSTEIN WR: Cellular communication through membrane junctions: Special considerations of wound healing and cancer. *Arch Intern Med* 129:299–305, 1972
42. AKISAKA T, YAMAMOTO T, GAY CV: Ultracytochemical investigation of calcium activated adenosine triphosphatase (Ca^{++}-ATPase) in chick tibia. *J Bone Miner Res* 3:19–25, 1988
43. NEUMAN WF, NEUMAN MW: *The Chemical Dynamics of Bone Mineral*. Chicago, The University of Chicago Press, 1958, pp. 101–136
44. ROBINSON RA: Observations regarding compartments for tracer calcium in the body, in *Bone Biodynamics*, edited by FROST HM, Boston, Little Brown, 1964, pp. 423–434
45. TIMMINS PA, WALL JC: Bone water. *Calcif Tissue Res* 23:1–5, 1977
46. MUELLER KH, TRIAS A, RAY RD: Bone density and composition: Age related and pathological changes in water and mineral content. *J Bone Joint Surg* 48-A:140–148, 1966
47. NEUMAN MW, NEUMAN WF: On the measurement of water compartments, pH and gradients in calvaria. *Calcif Tiss Int* 31:135–145, 1980
48. ROBINSON RA, ELLIOT SR: The water content of bone. I. The mass of water, inorganic crystals, organic matrix and "CO_2 space" components in a unit volume of dog bone. *J Bone Joint Surg* 39-A:167–188, 1957
49. ROBINSON RA, ELLIOT SR: The water content of bone. II. The division of bone water between the marrow-vascular-osteocyte space and the calcified osteoid matrix. *J Bone Joint Surgery* 40-A:1199–1210, 1958
50. BERENDSEN HJC, MIGCHELSEN C: Hydration structure of collagen and influence of salts. *Fed Proc* 25:998–1002, 1966
51. BERENDSEN HJC: Nuclear Magnetic Resonance study of collagen hydration. *J Chem Physics* 36:3297–3305, 1962
52. NEUMAN WF, NEUMAN MW: *The Chemical Dynamics of Bone Mineral*. Chicago, The University of Chicago Press, 1958, pp. 55–100
53. SWAN RC, PITTS RF: Neutralization of infused acid by nephrectomized dogs. *J Clin Invest* 34:205–212, 1955
54. SCHWARTZ WB, ORNING KJ, PORTER R: The internal distribution of hydrogen ions with varying degrees of metabolic acidosis. *J Clin Invest* 36:373–379, 1957
55. VAN SLYKE DD, CULLEN GE: Studies of acidosis: I. The bicarbonate concentration of the blood plasma: Its significance and its

determination as a measure of acidosis. *J Biol Chem* 30:289–346, 1917
56. ADLER S, ROY A, RELMAN AS: Intracellular acid-base regulation. I. The response of muscle cells to changes in CO_2 tension or extracellular bicarbonate concentration. *J Clin Invest* 44:8–20, 1965
57. BETTICE JA, GAMBLE JL: Skeletal buffering of acute metabolic acidosis. *Am J Physiol* 229:1618–1624, 1975
58. BROWN EB JR, GOOT B: Intracellular hydrogen ion changes and potassium movement. *Am J Physiol* 204:765–770, 1963
59. BROWN EB JR, KIM WG, MOORHEAD FA JR: Intgracellular pH during metabolic acidosis of intracellular and extracellular origin. *Proc Soc Exp Biol Med* 126:595–599, 1967
60. LEVITT MF, TURNER LB, SWEET AY, PANDIRI D: The response of bone, connective tissue and muscle to acute acidosis. *J Clin Invest* 35:98–105, 1956
61. POOLE-WILSON PA, CAMERON IR: Intracellular pH and K^+ of cardiac and skeletal muscle in acidosis and alkalosis. *Am J Physiol* 229:1305–1310, 1975
62. TOBIN RB: Plasma extracellular and muscle electrolyte responses to acute metabolic acidosis. *Am J Physiol* 186:131–138, 1956
63. BERGSTROM WH, RUVA FD: Changes in bone sodium during acute acidosis in the rat. *Am J Physiol* 198:1126–1128, 1960
64. BURNELL JM: In vivo response of muscle to changes in CO_2 tension or extracellular bicarbonate. *Am J Physiol* 215:1376–1383, 1968
65. FORBES GB, TOBIN RB, HARRISON A, MCCOORD A: Effect of acute hypernatremia, hyponatremia and acidosis on bone sodium. *Am J Physiol* 209:825–829, 1965
66. NORMAN N: The participation of bone in the sodium and potassium metabolism of the rat. II. The effect of variation of electrolyte intake, acidosis and alkalosis. *Acta Physiol Scand* 57:373–383, 1963
67. BARZEL US, JOWSEY J: The effects of chronic acid and alkali administration on bone turnover in adult rats. *Clin Sci* 36:517–524, 1969
68. BURNELL JM: Changes in bone sodium and carbonate in metabolic acidosis and alkalosis in the dog. *J Clin Invest* 50:327–331, 1971
69. LEMANN J JR, LITZOW JR, LENNON EJ: Studies of the mechanism by which chronic metabolic acidosis augments urinary calcium excretion in man. *J Clin Invest* 46:1318–1328, 1967
70. BUSHINSKY DA: Effects of parathyroid hormone on net proton flux from neonatal mouse calvariae. *Am J Physiol* 252:F585–F589, 1987
71. BUSHINSKY DA, GOLDRING JM, COE FL: Cellular contribution to pH-mediated calcium flux in neonatal mouse calvariae. *Am J Physiol* 248:F785–F789, 1985
72. BUSHINSKY DA, KRIEGER NS, GEISSER DI, GROSSMAN EB, COE FL: Effects of pH on bone calcium and proton fluxes in vitro. *Am J Physiol* 245:F204–F209, 1983
73. BUSHINSKY DA, LEVI-SETTI R, COE FL: Ion microprobe determination of bone surface elements: Effects of reduced medium pH. *Am J Physiol* 250:F1090–F1097, 1986
74. BUSHINSKY DA: Net proton influx into bone during metabolic but not respiratory acidosis. *Am J Physiol* 254:F306–F310, 1988
75. SCHAEFER KE, NICHOLS G JR, CAREY CR: Calcium phosphorus metabolism in man during acclimatization to carbon dioxide. *J Appl Physiol* 18:1079–1084, 1963
76. SCHAEFER KE, PASQUALE S, MESSIER AA, SHEA M: Phasic changes in bone CO_2 fractions, calcium and phosphorus during chronic hypercapnia. *J Appl Physiol* 48:802–811, 1980
77. POSNER A: Crystal chemistry of bone mineral. *Physiol Rev* 49: F60–F92, 1962
78. SKIPPER HE, NOLAN C, SIMPSON L: Studies on the hazard involved in use of ^{14}C III. Long term retention in bone. *J Biol Chem* 189:156–159, 1951
79. PELLEGRINO ED, BLITZ RM, ROGERS PJ: Bone carbonate and the double salt hypothesis: Its chemical, physical and physiological implications. *Trans Am Clin Climatol Assoc* 76:181–191, 1965
80. NEUMAN WF, MULRYAN BJ: Synthetic hydroxyapatite crystals. III. The carbonate system. *Calif Tiss Res* 1:94–104, 1967
81. POYART CF, BURSAUX E, FREMINET A: The bone CO_2 compartment: Evidence for a bicarbonate pool. *Resp Physiol* 25:89–99, 1975
82. PAYART CF, FREMINET A, BURSAUX E: The exchange of bone CO_2 in vivo. *Resp Physiol* 25:101–107, 1975
83. IRVING L, CHUTE AL: The participation of the carbonates of bone in the neutralization of ingested acid. *J Cell Comp Physiol* 2:157–176, 1932
84. GOTO K: Mineral metabolism in experimental acidosis. *J Biol Chem* 36:355–362, 1918
85. LOGAN MA, TAYLOR HL: Solubility of bone salt. II. Factors affecting its formation. *J Biol Chem* 125:377–392, 1938
86. BETTICE JA: Skeletal carbon dioxide stores during metabolic acidosis. *Am J Physiol* 247:F326–F330, 1984
87. BUSHINSKY DA, LECHLEIDER RJ: Mechanism of proton induced bone-calcium release: Calcium carbonate dissolution. *Am J Physiol* 253:F998–F1005, 1987
88. PASQUALE S, MESSIER AA, SHEA ML, SCHAEFER KE: Bone CO_2 titration curves in acute hypercapnia obtained with a modified titration technique. *J Appl Physiol* 48:197–201, 1980
89. BERGSTROM WH, WALLACE WM: Bone as a sodium and potassium reservoir. *J Clin Invest* 33:867–873, 1954
90. EDELMAN IS, JAMES AH, BADEN H, MOORE FD: Electrolyte composition of bone and the penetration of radiosodium and deuterium oxide into dog and human bone. *J Clin Invest* 33:127–131, 1954
91. HARTSUCK JM, JOHNSON JE, MOORE FD: Potassium in bone: Evidence for a nonexchangeable fraction. *Metabolism* 18:33–37, 1969
92. BERGSTROM WH, BELL EH: Bone magnesium content in normal and acidotic rats. *J Bone Joint Surg* 42-A:437–447, 1960
93. ALFREY AC, MILLER NL, TROW R: Effect of age and magnesium depletion on bone magnesium pools in rats. *J Clin Invest* 54:1074–1081, 1974
94. NICHOLS G, NICHOLS N JR: The availability of bone sodium. *Clin Res Proc* 1:91, 1953
95. PANDOLFO L, RECINE A, GEMELLI M, FAMA M: Effects of acidosis and alkalosis on sodium and potassium metaboism in bone. *Biochim Appl* 15:53–60, 1968
96. BUSHINSKY DA: Net calcium efflux from live bone during chronic metaboic but not respiratory acidosis. *Am J Physiol* 256:F836–F842, 1989
97. PELLEGRINO ED, BILTZ RM: The composition of human bone in uremia. Observations on the reservoir functions of bone and demonstration of a labile fraction of bone carbonate. *Medicine* 44:397–418, 1965
98. LEMANN J JR, LENNON EJ, GOODMAN AD, LITZOW JR, RELMAN AS: The net balance of acid in subjects given large loads of acid or alkali. *J Clin Invest* 44:507–514, 1965
99. LITZOW JR, LEMANN J JR, LENNON EJ: The effect of treatment of acidosis on calcium balance in patients with chronic azotemic renal disease. *J Clin Invest* 46:280–286, 1967
100. REIDENBERG MM, HOAG BL, CHANNICK BJ, SHUMAN CR, WILSON TGG: The response of bone to metabolic acidosis in man. *Metabolism* 15:236–241, 1966
101. GOODMAN AD, LEMANN J JR, LENNON EJ, RELMAN AS: Production, excretion, and net balance of fixed acid in patients with renal acidosis. *J Clin Invest* 44:495–506, 1965
102. DOMINGUEZ JH, RAISZ LG: Effects of changing hydrogen ion, carbonic acid and bicarbonate concentrations on bone resorption in vitro. *Calcif Tiss Int* 29:7–13, 1979
103. GOLDHABER P, RABADJIJA L: H^+ stimulation of cell-mediated bone resorption in tissue culture. *Am J Physiol* 253:E90–E98, 1987
104. KRAUT JA, MISHLER DR, KUROKAWA K: Effect of colchicine and calcitonin on calcemic response to metabolic acidosis. *Kidney Int* 25:608–612, 1984
105. KRAUT JA, MISHLER DR, SINGER FR, GOODMAN WG: The effects of metabolilc acidosis on bone formation and bone resorption in the rat. *Kidney Int* 30:694–700, 1986
106. CHAN VL, SAVDIE E, MASON RS, POSEN S: The effect of metabolic acidosis on vitamin D metabolites and bone histology in uremic rats. *Calcif Tiss Int* 37:158–164, 1985
107. BAYLINK D, WERGEDAL J, STAUFFER M: Formation, mineraliza-

tion and resorption of bone in hypophosphatemic rats. *J Clin Invest* 50:2519–2530, 1971
108. COBURN JW, MASSRY SG: Changes in serum and urinary calcium during phosphate depletion: Studies on mechanisms. *J Clin Invest* 49:1073–1087, 1970
109. GOLD LW, MASSRY SG, ARIEFF AI, COBURN JW: Renal bicarbonate wasting during phosphate depletion. A possible cause of altered acid-base homeostasis in hyperparathyroidism. *J Clin Invest* 52:2556–2562, 1973
110. EMMETT M, GOLDFARB S, AGUS ZS, NARINS RG: The pathophysiology of acid-base changes in chronically phosphate depleted rats. Bone-kidney interactions. *J Clin Invest* 59:291–298, 1977
111. BARON R, NEFF L, LOUVARD D, COURTOY PJ: Cell mediated extracellular acidification and bone resorption: Evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein at the osteoclast ruffled border. *J Cell Biol* 101:2210–2222, 1985
112. TETI A, BLAIR HC, TEITELBAUM SL, KAHN AJ, KOZIOL C, KONSEK J, ZAMBONIN-ZALLONE A, SCHLESINGER P: Cytoplasmic pH regulation and chloride/bicarbonate exchange in avian osteoclasts. *J Clin Invest* 83:227–233, 1989
113. TETI A, BLAIR HC, SCHLESINGER P, GRANO M, ZAMBONIN-ZALLONE A, KAHN AJ, TEITELBAUM SL, HRUSKA KA: Extracellular protons acidify osteoclasts, reduce cytosolic calcium, and promote expression of cell-matrix attachment structures. *J Clin Invest* 84:773–780, 1989
114. VAES G: Cellular biology and biochemical mechanism of bone resorption: A review of recent developments on the formation, activation and mode of action of osteoclasts. *Clin Orthop* 231:239–271, 1988
115. YAMAGUCHI DT, HAHN TJ, BEEKER TG, KLEEMAN CR, MAULLEM S: Relationship of cAMP and calcium messenger systems in prostaglandin-stimulated UMR-106 cells. *J Biol Chem* 263:10745–10753, 1988
116. YAMAGUCHI DT, HAHN TJ, KLEIN AE, KLEEMAN CR, MUALLEM S: Parathyroid hormone activated calcium channels in an osteoblast-like clonal osteosarcoma cell line: cAMP dependent and cAMP independent calcium channels. *J Biol Chem* 262:7711–7718, 1987
117. GREEN J, YAMAGUCHI DT, KLEEMAN CR, MUALLEM S: Cytosolic pH regulation in osteoblasts: Interaction of Na^+ and H^+ with the extracellular and intracellular faces of the Na^+/H^+ exchanger. *J Gen Physiol* 92:239–261, 1988
118. GREEN J, YAMAGUCHI DT, KLEEMAN CR, MUALLEM S: Cytosolic regulation in osteoblasts: Regulation of anion exchange by intracellular pH and Ca^{++} ions. *J Gen Physiol* 95:121–145, 1990
119. ARNETT TR, DEMPSTER DW: Effect of pH on bone resorption by rat osteoclasts in vitro. *Endocrinology* 119:119–124, 1986
120. LAU K, NICHOLS FR, TANNEN RL: Renal excretion of divalent ions in response to chronic acidosis: Evidence that systemic pH is not the controlling variable. *J Lab Clin Med* 109:27–33, 1987
121. HARAMATI A, NIENHUIS D: Renal handling of phosphate during acute respiratory acidosis and alkalosis in the rat. *Am J Physiol* 247:F596–F601, 1984
122. WEBB RK, WOODHALL PB, TISHER CC, GLAUBIGER G, NEELON FA, ROBINSON RR: Relationship between phosphaturia and acute hypercapnia in the rat. *J Clin Invest* 60:829–837, 1977
123. HANSEN AC, WAMBERG S, ENGEL K, KILDEBERG P: Balance of net base in the rat: Adaptation to and recovery from sustained hypercapnia. *Scand J Clin Lab Invest* 39:723–730, 1979
124. GRAY SP, MORRIS JEW, BROOKS CJ: Renal handling of calcium, magnesium, inorganic phosphate and hydrogen ions during prolonged exposure to elevated carbon dioxide concentrations. *Clin Sci Mol Med* 45:751–764, 1973
125. SILBERG BW, CALDER D, CARTER N, SELDIN D: Urinary calcium excretion in parathyroidectomized rats during metabolic and respiratory acidosis. (abstract) *Clin Res* 12:50, 1969
126. SCHAEFER KE: Effects of increased ambient CO_2 levels on human and animal health. *Experientia Basel* 38:1163–1168, 1982
127. KAPLAN EL, HILL BJ, LOCKE S, TOTH DN, PESKIN GW: Metabolic acidosis and parathyroid hormone secretion in sheep. *J Lab Clin Med* 78:819–822, 1971
128. MARTIN KJ, FREITAG JJ, BELLORIN-FONT E, CONRADES MB, KLAHR S, SLATOPOLSKY E: The effect of acute acidosis on the uptake of parathyroid hormone and the production of adenosine 3′5′ monophosphate by isolated perfused bone. *Endocrinology* 106:1607–1611, 1980
129. WACHMAN A, BERNSTEIN DS: Parathyroid hormone in metabolic acidosis. *Clin Orthop* 69:252–263, 1970
130. BECK N, WEBSTER SK: Effects of acute metabolic acidosis on parathyroid hormone action and calcium mobilization. *Am J Physiol* 230:127–135, 1976
131. COE FL, FIRPO JJ, HOLLANDSWORTH DL, SEGIL L, CANTERBURY JM, REISS EM: Effect of acute and chronic metabolic acidosis on serum immunoreactive parathyroid hormone in man. *Kidney Int* 8:262–273, 1975
132. FRALEY DS, ADLER S: An extrarenal role for parathyroid hormone in the disposal of acute acid loads in rats and dogs. *J Clin Invest* 63:985–997, 1979
133. ARRUDA JAL, ALLA V, RUBINSTEIN H, CRUZ-SOTO M, SABATINI S, BATLLE DC, KURTZMAN NA: Parathyroid hormone and extrarenal acid buffering. *Am J Physiol* 239:F533–F538, 1980
134. MADIAS NE, JOHN CA, HAMER SM: Independence of the acute acid buffering response from endogenous parathyroid hormone. *Am J Physiol* 243:F141–F149, 1982
135. ADAMS ND, GRAY RW, LEMANN J: The calciuria of increased fixed acid production in humans: Evidence against a role for parathyroid hormone and $1,25(OH)_2$D-vitamin D. *Calcif Tissue Int* 28:233–238, 1979
136. WEBER HP, GRAY RW, DOMINGUEZ JH, LEMANN J JR: The lack of effect of chronic metabolic acidosis on 25-OH vitamin D metabolism and serum parathyroid hormone in humans. *J Clin Endocrinol Metab* 43:1047–1055, 1976
137. JAFFE HL, BODANSKY A, CHANDLER JP: Ammonium chloride decalcification, as modified by calcium intake: The relation between generalized osteoporosis and ostitis fibrosa. *J Exp Med* 56:823–834, 1932
138. DELLING G, DONATH K: Morphometrische, elektronen-mikroskopische and physikalisch-chemische. Untersuchungen uber die experimentelle osteoporose bei chromischer Acidose *Virchow Arch (A) Pathol Anat* 358:321–330, 1973
139. NEWELL GK, BEAUCHENE RE: Effects of dietary calcium level, acid stress, and age on renal, serum, and bone responses of rats. *J Nutr* 105:1039–1047, 1975
140. UPTON PK, L'ESTRANGE JL: Effects of chronic hydrochloric and lactic acid administrations on food intake, blood acid-base balance and bone composition of the rat. *Quart J Exp Physiol* 62:223–235, 1977
141. KRISHNARAO GVG, DRAPER HH: Age related changes in the bones of adult mice. *J Gerontol* 24:149–151, 1969
142. KAYE M: The effect in the rat of varying intakes of dietary calcium, phosphorus and hydrogen ion on hyperparathyroidism due to chronic renal failure. *J Clin Invest* 53:256–260, 1974
143. CHAN YL, ALFREY AC, POSEN S, LISSNER D, HILLS E, DUNSTAN CR, EVANS RA: The effect of aluminum on normal and uremic rats: tissue distribution, vitamin D metabolites and quantitative bone histology. *Calcif Tissue Int* 39:344–351, 1983
144. ALBRIGHT F, BURNETT CH, PARSON W, REIFENSTEIN EC JR, ROOS A: Osteomalacia and late rickets. *Medicine* 25:399–479, 1946
145. MAULTEN C, MONTOREANO R, LABARRERE C: Early skeletal effect of alkali therapy upon the osteomalacia of renal tubular acidosis. *J Clin Endocrinol Metab* 42:875–881, 1976
146. PINES KL, MUDGE GH: Renal tubular acidosis with osteomalacia: Report of three cases. *Am J Med* 11:302–311, 1951
147. LEE DBN, DRINKARD JP, ROSEN VJ, GONICK HC: The adult Fanconi syndrome: Observatoins on etiology, morphology, renal function and mineral metabolism in three patients. *Medicine* 51:107–138, 1972
148. MCSHERRY E: Renal tubular acidosis in childhood (Nephrology Forum). *Kidney Int* 20:799–809, 1981
149. BRENNER RJ, SPRING DB, SEBASTIAN A, MCSHERRY EM, GENANT HK, PALUBINSKAS AJ, MORRIS RC JR: Incidence of radiographically evident bone disease, nephrocalcinosis, and nephrolithiasis in various types of renal tubular acidosis. *N Engl J Med* 307:217–221, 1982
150. MCSHERRY E, MORRIS RC JR: Attainment and maintenance of

normal stature with alkali therapy in infants and children with classic renal tubular acidosis. *J Clin Invest* 61:509–527, 1978
151. McSherry E: Acidosis and growth in nonuremic renal disease. *Kidney Int* 14:349–354, 1978
152. Albright F, Consolazio WV, Coombs FS, Sulkowith HW, Talbott JH: Metabolic studies and therapy in a case of nephrocalcinosis with rickets and dwarfism. *Bull Johns Hopkins J* 66:7–14, 1940
153. Cooke RE, Kleeman CR: Distal tubular dysfunction with renal calcification. *Yale J Biol Med* 23:199–209, 1950
154. Royer P: Chronic tubular disease, (Chapter 17) in *Nephrology*, edited by Hamburger J, Philadelphia, W.B. Saunders Co., 1968, pp. 597–601
155. Sobel E: Preschool and school age child: *Growth Problems in Biological Basis of Pediatric Practice*, New York, McGraw-Hill (vol 2), 1968, pp. 1568–1569
156. Nash MA, Torrado AD, Greifer I, Spitzer A, Edelmann CM Jr: Renal tubular acidosis in infants and children. *J Pediatr* 80:738–743, 1972
157. Watson EH, Lowrey GH: *Growth and Development of Children*. Chicago, Year Book of Medical Publishers, Inc., 1969, pp. 87–88
158. Palmer RH, Cornfeld D: Primary renal tubular acidosis recognized and treated at three days of age. A case report illustrating the value of routine postmortem examination. *Clin Pediatr* 12:140–144, 1973
159. Seldin DW, Wilson JD: Renal tubular acidosis, in *The Metabolic Basis of Inherited Disease*, edited by Stanbury JB, Wyngaarden JB, Fredrickson DS, New York, McGraw-Hill Book Company, (3rd ed) 1972, pp. 1548–1566
160. Cunningham J, Fraher LJ, Clemens TL, Revell PA, Papapoulos SE: Chronic acidosis with metabolic bone disease: Effect of alkali on bone morphology and vitamin D metabolism. *Am J Med* 73:199–204, 1982
161. Richards P, Chamberlain MJ, Wrong OM: Treatment of osteomalacia of renal tubular acidosis by sodium bicarbonate alone. *Lancet* 2:994–997, 1972
162. Bikle DD, Rasmussen H: The ionic control of 1,25 dihydroxyvitamin D_3 production in isolated chick renal tubules. *J Clin Invest* 55:292–298, 1975
163. Sauveur B, Garabedian M, Fellot C, Mongin P, Balsan S: The effect of induced metabolic acidosis on vitamin D_3 metabolism in rachitic chicks. *Calcif Tissue Res* 23:121–124, 1977
164. Baran DT, Lee SW, Jo OD, Avioli LV: Acquired alterations in vitamin D metabolism in the acidotic state. *Calcif Tiss Int* 34:165–168, 1982
165. Lee SW, Russell J, Avioli LV: 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol conversion impaired by systemic metabolic acidosis. *Science* 195:994–996, 1977
166. Reddy GS, Jones G, Kooh SW, Fraser D: Inhibition of 25-hydroxyvitamin D_3-1 hydroxylase by chronic metabolic acidosis. *Am J Physiol* 243:E265–E271, 1982
167. Kawashima H, Kraut JA, Kurokawa K: Metabolic acidosis suppresses 25-hydroxyvitamin D_3-1 hydroxylase in the rat kidney. *J Clin Invest* 70:135–140, 1982
168. Cunningham J, Bikle DD, Avioli LV: Acute but not chronic metabolic acidosis disturbs 25-hydroxyvitamin D_3 metabolism. *Kidney Int* 25:47–52, 1984
169. Bushinsky DA, Riera GS, Favus MJ, Coe FL: Response of serum $1,25(OH)_2D_3$ to variation of ionized calcium during chronic acidosis. *Am J Physiol* 249:F361–F365, 1985
170. Gafter U, Kraut JA, Lee DBN, Silis V, Walling MW, Kurokawa K, Hausler MR, Coburn JW: Effect of metabolic acidosis on intestinal absorption of calcium and phosphorus. *Am J Physiol* 239:G480–G484, 1980
171. Kraut JA, Gordon EM, Ransom JC, Horst R, Slatopolsky E, Coburn JW, Kurokawa K: Effect of chronic metabolic acidosis on vitamin D metabolism in humans. *Kidney Int* 24:644–648, 1983
172. Chesney RW, Kaplan BS, Phelps M, DeLuca HF: Renal tubular acidosis does not alter circulating values of calcitriol ($1,25(OH)_2D$). (abstract) *Am Soc Bone Miner Res* Fifth Annual Scientific meeting, San Antonio, June, 1983, A 31
173. Lucas RC: Form of late rickets associated with albuminuria, rickets of adolescents. *Lancet* 1:993–1015, 1883
174. Fletcher HM: Case of infantilism with polyuria and chronic renal disease. *Proc Roy Soc Med* 4:95–112, 1911
175. Barber H: Renal dwarfism. *Quart J Med* 14:205–217, 1920
176. Parsons LG: The bone changes occuring in renal and coeliac infantilism and their relation to rickets. I. Renal rickets. *Arch Dis Child* 2:1–25, 1927
177. Teall CG: A radiological study of the bone changes in renal infantilism. *Brit J Radiol* 1:47–49, 1928
178. Langmead FS, Orr JW: Renal rickets associated with parathyroid hyperplasia. *Arch Dis Child* 8:265–274, 1933
179. Smyth FS, Goldman L: Renal rickets with metabolic calcification and parathyroid dysfunction. *Am J Dis Child* 48:597–610, 1934
180. Shelling DH, Remsen D: Renal rickets: Report of a case showing four enlarged parathyroids and evidence of parathyroid hypersecretion. *Bull John Hopkins Hosp* 57:158–171, 1935
181. Price NL, Davie TB: Renal rickets. *Br J Surg* 24:548–565, 1936
182. Derow HA, Brodny ML: Congenital posterior urethral valve causing renal rickets. *N Engl J Med* 221:685–699, 1939
183. Magnus HA, Scott RB: Chronic renal destruction and parathyroid hyperplasia. *J Pathol Bacteriol* 42:665–685, 1936
184. Sherrard DJ: Renal osteodystrophy, in *Seminars in Nephrology*, edited by Kurtzman NA, 1986, pp. 56–67
185. Ginzler AM, Jaffe HL: Osseous findings in chronic renal insufficiency in adults. *Am J Pathol* 17:293–315, 1941
186. Albright F, Reifenstein EC: *The Parathyroid Glands and Metabolic Bone Disease*. Baltimore, Williams and Wilkins, 1948
187. Snapper I: *Bone Disease in Medical Practice*. New York, Grune and Stratton, 1957
188. Bartter FC: Metabolic bone disease, in *Metabolic Disturbances in Clinical Medicine*, edited by Smart GA, London, J and A Churchill, 1958
189. West CD, Smith WC: An attempt to elucidate the cause of growth retardation in renal disease. *Am J Dis Child* 91:460–476, 1956
190. Fletcher RF, Jones JH, Morgan DB: Bone disease in chronic renal failure. *Quart J Med* 32:321–339, 1963
191. Bergstrom WH, de-Leon AS, van Gemund JJ: Growth abberations in renal disease. *Pediatr Clin N Am* 11:563–575, 1964
192. Stanbury SW, Lumb GA: Metabolic studies of renal osteodystrophy. I. Calcium, phosphorus and nitrogen metabolism in rickets, osteomalacia and hyperparathyroidism complicating chronic uremia and in the osteomalacia of the adult Fanconi Syndrome. *Medicine* 41:1–31, 1962
193. Liu SH, Chu HI: Studies of calcium and phosphorus metabolism with special reference to pathogenesis and effects of dihydrotachysterol (A.T. 10) and iron. *Medicine* 22:103–161, 1943
194. Dent CE, Harper CM, Philpot GR: The treatment of renal-glomerular osteodystrophy. *Quart J Med* 30:1–18, 1961
195. Mitchell AG: Nephrosclerosis (chronic interstitial nephritis) in childhood with special reference to renal rickets. *Am J Dis Child* 40:101–145, 1930
196. Cochran M, Nordin BEC: Role of acidosis in renal osteomalacia. *Br Med J* 2:276–279, 1969
197. Ingham JP, Kleerekeper M, Stewart JH, Posen S: Symptomatic skeletal disease in non-terminal renal failure. *Med J Aust* 1:873–876, 1974
198. Cochran M, Wilkinson R: Effect of correction of metabolic acidosis on bone mineralization rates in patients with renal osteomalacia. *Nephron* 15:98–110, 1975
199. Bishop MC, Ledingham JG: Alkali treatment of renal osteodystrophy. *Br Med J* 4:529–534, 1972
200. Mora Palma FJ, Ellis HE, Cook DB, Dewar JH, Ward MK, Wilkinson R, Kerr DNS: Osteomalacia in patients with chronic renal failure before dialysis or transplantation. *Quart J Med* 52:332–348, 1983
201. Green J, Kleeman CR, Muallem S: Mode of regulation of Cl/HCO_3^- exchanger in UMR-106 cells: Role of Ca^{2+} calmodulin and cyclic AMP. (submitted for publication)