Regulation of bone cell function by acid–base balance

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Bone growth and turnover results from the coordinated activities of two key cell types. Bone matrix is deposited and mineralised by osteoblasts and it is resorbed by osteoclasts, multinucleate cells that excavate pits on bone surfaces. It has been known since the early 20th century that systemic acidosis causes depletion of the skeleton, an effect assumed to result from physico-chemical dissolution of bone mineral. However, our own work has shown that resorption pit formation by cultured osteoclasts was absolutely dependent on extracellular acidification; these cells are inactive at pH levels above about 7·3 and show maximum stimulation at a pH of about 6·9. Bone resorption is most sensitive to changes in H^+ concentration at a pH of about 7·1 (which may be close to the interstitial pH in bone). In this region pH shifts of $< 0·05$ units can cause a doubling or halving of pit formation. In whole-bone cultures, chronic HCO_3^- acidosis results in similar stimulations of osteoclast-mediated Ca^{2+} release, with a negligible physico-chemical component. *In vivo*, severe systemic acidosis (pH change of about −0·05 to −0·20) often results from renal disease; milder chronic acidosis (pH change of about −0·02 to −0·05) can be caused by excessive protein intake, acid feeding, prolonged exercise, ageing, airway diseases or the menopause. Acidosis can also occur locally as a result of inflammation, infection, wounds, tumours or diabetic ischaemia. Cell function, including that of osteoblasts, is normally impaired by acid; the unusual stimulatory effect of acid on osteoclasts may represent a primitive 'fail-safe' that evolved with terrestrial vertebrates to correct systemic acidosis by ensuring release of alkaline bone mineral when the lungs and kidneys are unable to remove sufficient H^+ equivalent. The present results suggest that even subtle chronic acidosis could be sufficient to cause appreciable bone loss over time.

Osteoclasts: Osteoblasts: Bone: Acid: pH

One of the fundamental problems faced by all multicellular organisms is dealing with the protons produced as a result of the metabolism of food by cells. The most basic function of the vasculature is to deliver food and O_2 to cells and to remove waste, including H^+ and CO_2, which are excreted via urine and expired air respectively. The skeletons of land vertebrates contain a massive reserve of base, which is ultimately available as a 'fail-safe' mechanism to buffer H^+ if the kidneys and lungs are unable to maintain acid–base balance within narrow physiological limits.

The deleterious effects of systemic acidosis on the skeleton have long been recognised (Goto, 1918; Jaffe *et al.* 1932). The present review will discuss the role of acid–base balance in skeletal homeostasis in the light of more recent discoveries concerning the actions of extracellular pH on bone cells.

Bone cell biology

'The skeleton, out of sight and often out of mind, is a formidable mass of tissue occupying about 9 % of the body by bulk and no less than 17 % by weight. The stability and immutability of dry bones and their persistence over the centuries, and even millions of years after the soft tissues have turned to dust, gives us a false idea of bone during life. Its fixity after death is in sharp contrast to its ceaseless activity during life' (Cooke, 1955*a,b*).

Bone is a connective tissue that consists principally of a mineralised extracellular matrix plus the specialised cells, osteoblasts, osteocytes and osteoclasts. The structural component of the organic phase is type I (fibrous) collagen, which comprises about 90 % of the bone protein; the inorganic phase is mainly tiny crystals of the alkaline mineral hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$.

Osteoblasts, the bone-forming cells, work in groups to secrete and then mineralise patches of new bone matrix. Histologically, active osteoblasts appear as plump cuboidal cells on the bone surface (Fig. 1), with the prominent rough endoplasmic reticulum characteristic of protein-secreting cells. Active osteoblasts express large amounts of alkaline phosphatase, which probably aids mineralisation by
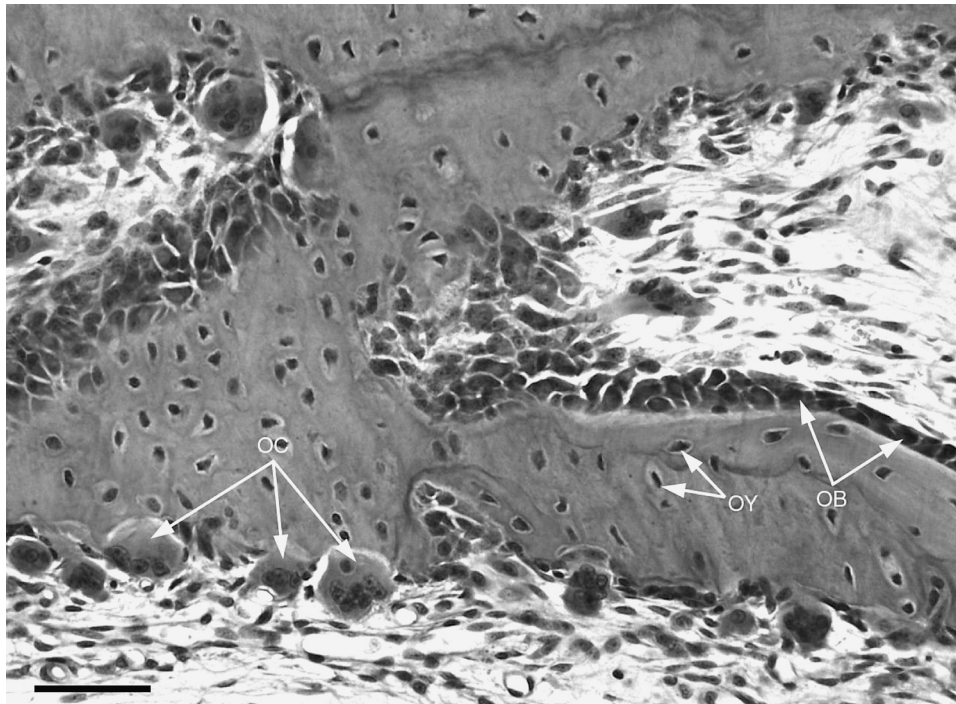
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Fig. 1. Section of rapidly-remodelling bone from the jaw of a young animal, showing active osteoblasts (OB) forming bone; osteocytes (OY) entombed in bone matrix; multinucleate osteoclasts (OC) forming resorption pits in the bone surface. Numerous fibroblast-like cells and capillaries are evident in the surrounding stroma. Scale bar 50 μm.

liberating inorganic phosphate. Quiescent bone surfaces are covered by a near-continuous single layer of flattened inactive osteoblasts, often referred to as 'bone-lining cells'. Osteoblasts are derived from mesenchymal progenitor cells that in the bone marrow, at least, are also capable of differentiating into adipocytes and fibroblasts. Osteoblasts, or their progenitors, express receptors for many hormones, including those for parathyroid hormone, 1,25-dihydroxy-cholecalciferol, sex steroids and corticosteroids; they are also responsive to, and may produce, a wide range of growth factors and cytokines. During bone formation some osteo-blasts become engulfed by the accumulating matrix around them and differentiate into osteocytes, the interconnected low-density network of cells that ramify throughout all living bone (Fig. 1). Osteocytes are thought to mediate the remarkable mechanical responsiveness of bone by functioning as strain sensors that communicate with cells (osteoblasts and osteoclasts) on bone surfaces.

The destruction of bone is accomplished by osteoclasts, large motile multinucleate cells of promonocytic origin that resorb characteristic 'scalloped' pits and trails in bone surfaces (Fig. 1). Osteoclasts are formed by the fusion of mononuclear promonocytic precursors present in the marrow and circulation. They form resorption pits by attaching tightly to the bone surface, secreting protons to dissolve the mineral phase, and proteolytic enzymes (chiefly cathepsin K) to degrade the collagenous matrix. Osteoclasts achieve a high surface area of interaction with the bone by means of a convoluted membranous organ, the so-called 'ruffled border'; the adjacent resorption space can be considered as a specialised extracellular lysosome. Osteoclasts express high

levels of tartrate-resistant acid phosphatase, the function of which is uncertain. Mature osteoclasts express receptors for calcitonin, a potent inhibitory hormone, and for prosta-glandins, but appear not to be directly responsive to most other hormones or growth factors.

During adult life the skeleton undergoes a continual process of repair and renewal. Bone remodelling is a surface phenomenon; the turnover rate in trabecular bone may be up to ten times greater than that in cortical bone, reflecting the large surface area presented by the former tissue. Mineralised bone matrix is resorbed by osteoclasts and replaced in plywood-like layers, or lamellae, by groups of osteoblasts. This sequence of events is tightly coordinated both temporally and spatially. Under normal circumstances in young adults remodelling activity keeps overall bone mass relatively constant. However, ageing, the menopause and many other pathophysiological states can alter the balance of the turnover process, such that resorption begins to outstrip formation, leading to net bone loss and ultimately osteoporosis. This outcome could be the result not only of enhanced osteoclastic resorption but also of reduced osteo-blastic function. Trabecular bone sites, for example, in the vertebral bodies (see Fig. 2) or in the ends of the long bones are particularly susceptible to remodelling imbalances, as a result of the relatively high turnover rate (about 25 % per year).

Acid–base balance

Because the machinery of cells is very sensitive to changes in H$^+$ concentration, precise maintenance of pH in the blood
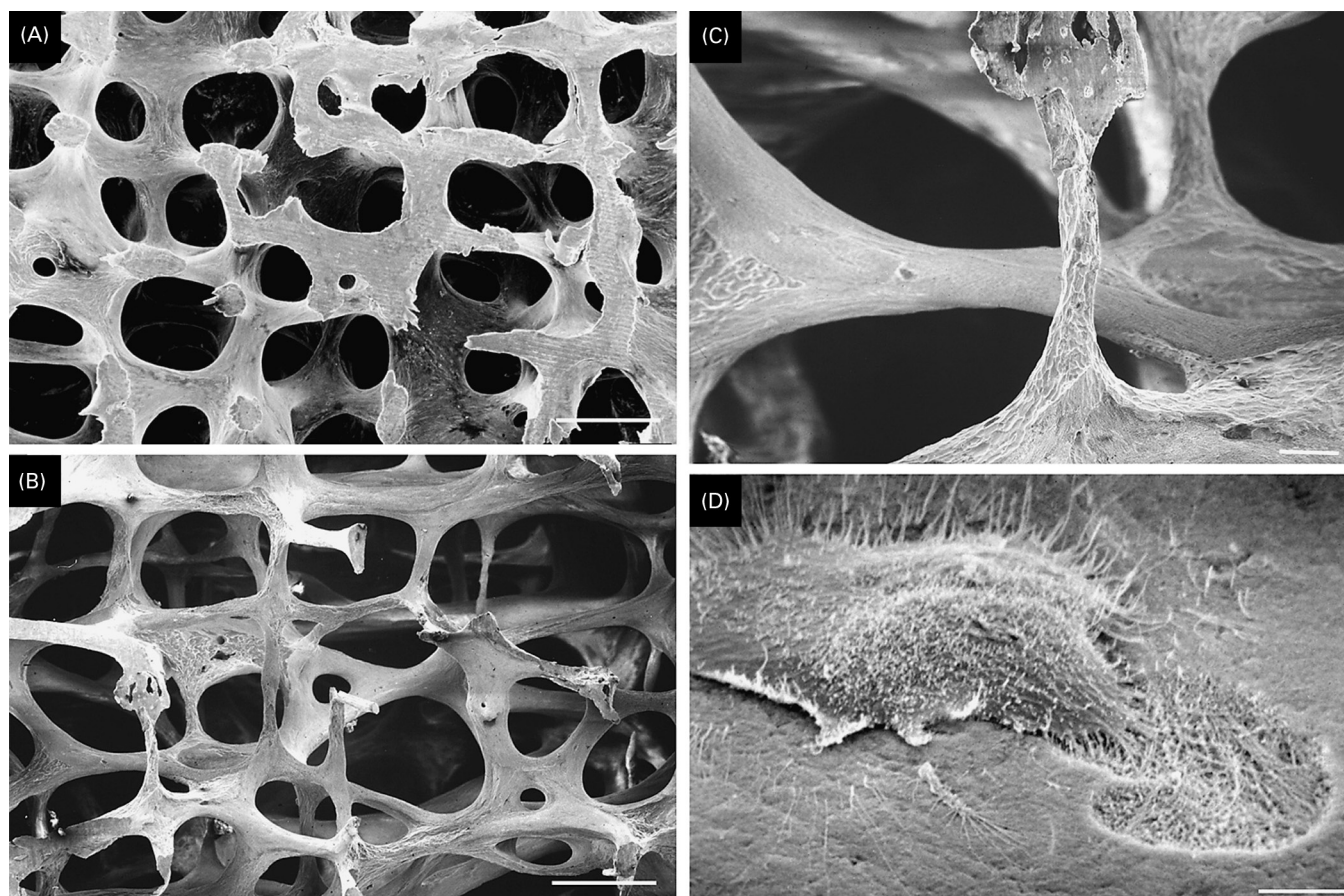
Fig. 2. (A, B), Scanning electron micrographs of vertical sections through the bodies of the third lumbar vertebrae (after removal of bone cells and soft tissues) of a normal 30-year-old woman and a 71-year-old woman, respectively. For the older woman severe osteoporotic changes are evident; the trabeculae are eroded to thin mechanically-insufficient rods. Scale bars 500 μm. (C), High power detail of micrograph B, showing extensive osteoclastic resorption pits on the vertical trabecular element in the foreground; this element displays a possible microfracture callus at the top. Scale bar 100 μm. (D), Scanning electron micrograph of an acid-activated rat osteoclast, with adjacent resorption pit, cultured on a slice of polished cow bone. Scale bar 10 μm.

and extracellular fluid is needed. Blood pH is buffered by plasma proteins, by the numerous histidine residues of haemoglobin and, most importantly, via the CO_2–HCO_3^- system. Addition of H^+ to the system will result in a pH decrease with a reduction of HCO_3^- levels without substantial alteration of the CO_2 concentration; this process is termed a 'metabolic acidosis'. H^+ generated in this way, together with associated waste anions, must be excreted via the kidneys to produce acidified urine. In renal disease, which is the major cause of chronic severe systemic acidosis, the ability of the kidneys to secrete H^+ into the urine (and to reabsorb HCO_3^-) is impaired or lost; arterial blood pH in severe renal metabolic acidosis may be as low as about 7·2. Conversely, addition of CO_2 to the system as a result of respiration causes an increase in H^+ concentration (i.e. pH reduction) without marked alteration of the HCO_3^- concentration. Normally, CO_2 is expelled via the lungs, but when this process does not occur to the necessary extent, usually because of chronic respiratory diseases or, acutely, due to severe exercise, a 'respiratory acidosis' results.

Systemic acidosis, albeit of a generally mild nature, may thus result from high-protein diets, ingestion of inorganic acids or their equivalents or simply as a result of declining renal, respiratory and vascular function with advancing age. Acidosis can also arise locally (i.e. at tissue level) as a result of reduced vascular supply due to inflammations, infections, tumours, wounds, diabetes or ageing. At the cellular level, a basic action of many growth factors and cytokines is to stimulate rapid H^+ efflux from cells, most simply as a result of increased cellular metabolism; the mitogens parathyroid hormone and insulin-like growth factor 1 exert similar effects on cultured osteoblasts (Barrett *et al.* 1997; Santhanagopal & Dixon, 1999) or whole bone (Belinsky & Tashjian, 2000).

Although the pH of arterial blood is normally about 7·40, and that of venous blood about 7·36, it is important to bear in mind that the pH of the extracellular fluid bathing cells is likely to be < 7·36 and subject to complex dynamic gradients, depending on the metabolic activity of the cells and their distance from the nearest capillary. In view of the obvious technical difficulties, this area has not been well investigated. Data are not available for bone, but in normal skin interstitial pH has been measured at about 7·1 (Martin & Jain, 1994).

Action of acidosis on bone *in vivo*

It was noted >80 years ago that acid feeding in rabbits resulted in skeletal depletion (Goto, 1918). Following the suggestion of Albright & Reifenstein (1948), Ca^{2+} balance studies provided evidence that the skeleton could play a homeostatic role in buffering acid loads (Lemann *et al.* 1966; Relman, 1968). Systemic acidosis probably contributes directly to renal osteodystrophy, including the osteomalacic component of this disease (Avioli, 1978; Cunningham *et al.* 1982). However, the contribution made by acidosis to bone loss in chronic human kidney disease has been difficult to evaluate in most studies because of the resulting perturbations in the 1,25-dihydroxycholecalciferol–parathyroid hormone axis.

Studies of experimental acidosis in rats (achieved by feeding NH_4Cl, which leaves a residue of HCl after metabolism and excretion of the nitrogenous component) indicated that osteoclastic bone resorption was increased, causing osteoporosis (Barzel & Jowsey, 1969; Chan *et al.* 1985). Moreover, in thyroparathyroidectomised rats, acute NH_4Cl-induced HCO_3^- acidosis was shown to result in a striking hypercalcaemia that was prevented by calcitonin or colchicine, implying osteoclast involvement (Kraut *et al.* 1984; see also Arnett & Dempster, 1990). However, despite these findings, and the results summarised later, the assumption has persisted that bone would buffer an acid load primarily via physico-chemical release of alkaline bone mineral, and that the skeleton thus acts as a 'giant ion-exchange column' (Green & Kleeman, 1991; Barzel, 1995).

Effects of pH on bone cells

Osteoclasts

The direct effects of pH on bone resorption were discovered when it was found that raising ambient CO_2 levels caused osteoclasts, isolated from fragmented rat bones and then cultured on polished cow bone slices, to excavate character-istic resorption pits. To investigate whether this effect was due to changes in H^+ concentration, osteoclasts were cultured overnight in low-HCO_3^- media without CO_2, using an artificial buffering system to maintain a range of pH values between 6·8 and 7·4. Osteoclasts were observed to be almost inactive at pH 7·4, which corresponds to 'physiological' or blood pH, but resorption pit formation increased steeply as pH was reduced (Arnett & Dempster, 1986, 1987). Surprisingly, osteoclasts resorb bone efficiently even at pH 6·3–6·4, although their survival may be somewhat reduced (Murrills *et al.* 1993). Subsequent short-term experiments using physiological CO_2–HCO_3^- buffering indicated that osteoclasts are particularly sensitive to pH changes at approximately pH 7·1, such that pH reductions of only a few hundredths of a unit caused a doubling of resorption pit formation; below a pH of about 7·0, the stimulatory effect begins to plateau (Arnett & Spowage, 1996). Whether the extracellular pH adjacent to osteoclasts in bone is approximately 7·1, as is the case for skin (Martin & Jain, 1994), remains to be determined. This acid-activation response has been observed in all species studied to date (Figs. 3–5). Importantly, this is not a short-term effect from which tachyphyllaxis (or 'escape') occurs, as is often the case for hormone-mediated phenomena. Longer-term cultures show that acid-activated osteoclasts continue to form resorption pits over periods of ≥7 d, magnifying the effects of modest pH differences (Fig. 6).

Further studies have shown that osteoclast activation can be considered as a two-step process. The initial 'switching on' of resorption requires acidification of the extracellular environment of the osteoclast to pH <7·2; further stimulation by other factors can then occur (for example, see Fig. 7). Clearly, a very wide range of hormones, cytokines, growth factors and other agents are known to exert a stimulatory action on bone resorption. Some agents such as parathyroid hormone, 1,25-dihydroxycholecalciferol, prostaglandins and interleukin 1 have long been recognised,
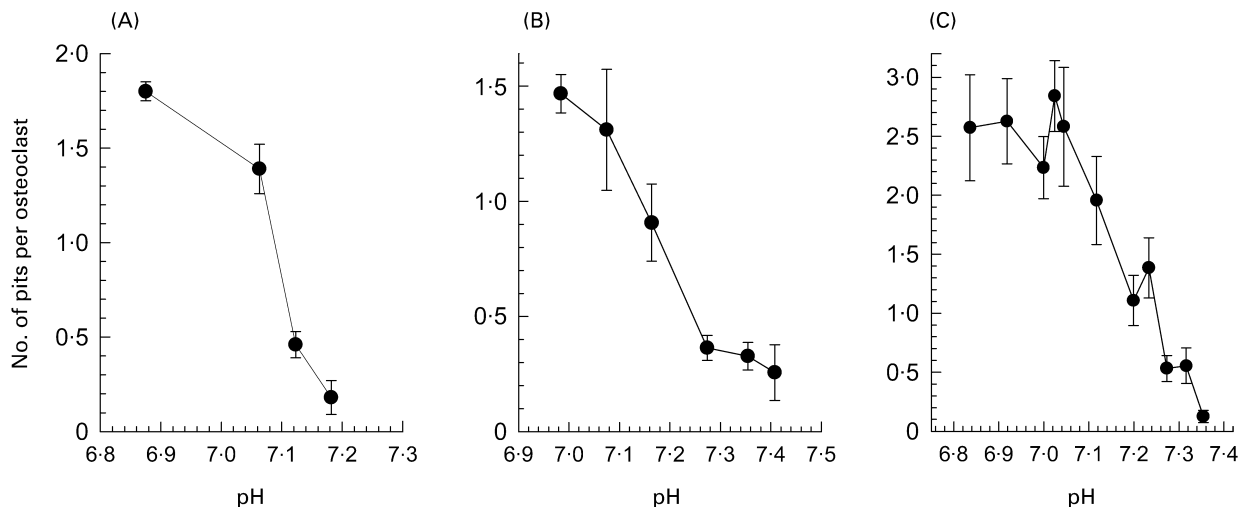

Fig. 3. Acid activation of resorption pit formation by rat (A), chick (B), and human osteoclastoma-derived osteoclasts (C) cultured 24 h on polished ivory discs (●——●). Culture medium was buffered by CO_2/HCO_3^- and pH was adjusted by addition of HCl or NaOH; pH measurements were made by blood gas analysers. Values are means with their standard errors represented by vertical bars for five determinations.
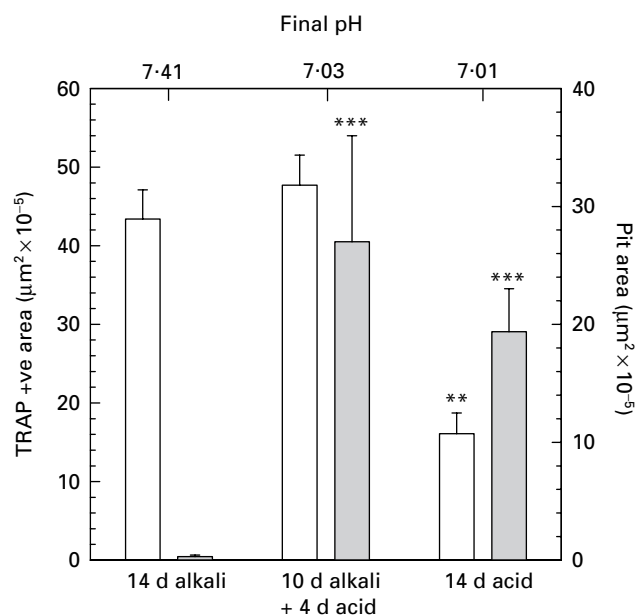
Fig. 4. Effects of pH variations on osteoclast formation (□) and resorption (▨) in mouse marrow cultures. Incubation of mouse marrow cultures in control medium at blood pH (7·41) for 14 d resulted in abundant tartrate-resistant acid phosphatase-positive (TRAP +ve) multinucleated osteoclast formation, but almost no resorption. In cultures maintained in alkaline medium for 10 d, followed by 4 d in acidified medium, formation of TRAP-positive osteoclasts was similar to that at 14 d at pH 7·41 but resorption pit formation was increased 93-fold. Continuous incubation in acidified media (pH 7·01) for 14 d reduced TRAP-positive multinucleate cell formation, but further increased the pit area:TRAP-positive area. Values are means with their standard errors represented by vertical bars. Significantly different from the 14 d alkaline medium values: **$P < 0.01$, ***$P < 0.001$. (Reproduced by courtesy of Dr M. Morrison.)

based on investigations both *in vivo* and *in vitro*; other agents such as receptor activator of nuclear factor kappa B ligand (RANKL also termed osteoprotegerin ligand; Lacey *et al.* 1998) and purine nucleotides (Morrison *et al.* 1998; Hoebertz *et al.* 2001) were discovered more recently. However, the critical interaction of osteolytic agents with pH (illustrated in Figs. 7 and 8) has not always been appreciated.

In contrast, the formation of osteoclasts from marrow precursors is not stimulated by reduced extracellular pH, and may even show slight inhibition (Morrison & Arnett, 1998). However, osteoclasts formed in long-term mouse marrow cultures at blood pH (7·4) show no resorptive activity until stimulated by acidification (Fig. 4).

Osteoblasts

Studies by Bushinsky and colleagues (Bushinsky, 1995; Frick & Bushinsky, 1998) have shown that the negative impact of acidification on bone may not be confined to pro-resorptive effects. Cultured osteoblasts show reduced collagen synthesis and mineralisation when pH is reduced. Our own observations indicate that the formation of mineralised bone nodules in long-term osteoblast cultures is completely blocked by acidification to pH 6·9 (Fig. 9).
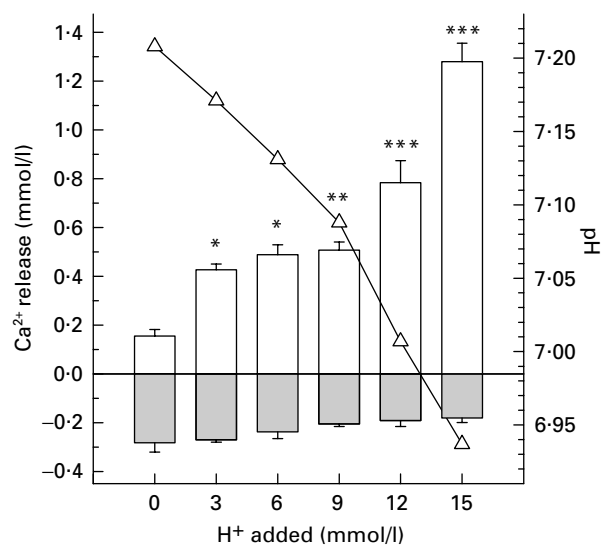

Fig. 5. Stimulatory effect of small decreases in medium pH(△–△), achieved by adding hydrogen ions (i.e. HCO_3^- acidosis) as hydrochloric acid, on calcium ion release from live mouse half-calvaria cultured for 3 d (□). In dead bones, killed by freeze thawing (▨), a net calcium ion influx occurred which was slightly reduced as pH decreased. Values are means with their standard errors represented by vertical bars for five determinations. Significantly different from the non-acidified control values: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Whole bone

The previously mentioned cell-culture studies demonstrated conclusively that acidosis stimulates osteoclasts to form three-dimensional resorption pits on mineralised tissue surfaces, a process that involves removal of both mineral and collagenous organic matrix. Whole-bone organ-culture experiments have been used to address the question of whether chronic acidosis stimulates Ca^{2+} efflux and bone depletion *in vivo* by physico-chemical or cell-mediated means. The work of Bushinsky and colleagues (Bushinsky *et al.* 1983, 1985; Bushinsky & Lechleider, 1987) using cultured mouse calvarial bones indicated that during the first few hours of exposure to acidosis there was a physico-chemical release of Ca^{2+} from bone coupled to a large net influx of H^+ into bone. After 2 d exposure to acidosis a cell-mediated component of Ca^{2+} release from calvaria was also detected (Bushinsky, 1989, 1995). Other studies from the same group reported that HCO_3^- acidosis was a more effective stimulator of Ca^{2+} release from bone than CO_2 acidosis (Bushinsky, 1989, 1995; Bushinsky *et al.* 1992). Work from another group (Goldhaber & Rabadjija, 1987; Rabadjija *et al.* 1990) indicated that Ca^{2+} efflux from calvarial bones subjected to HCO_3^- acidosis was almost entirely dependent on osteoclastic resorption, since the effect was completely blocked by calcitonin, the specific inhibitory hormone for osteoclasts. Our own experiments (Figs. 5 and 10) confirmed this finding and showed that HCO_3^- acidosis stimulates resorption of cultured mouse calvarial bones in a similar manner to that observed for mature osteoclast cultures (Meghji *et al.* 2001); the sensitivity of these bones is
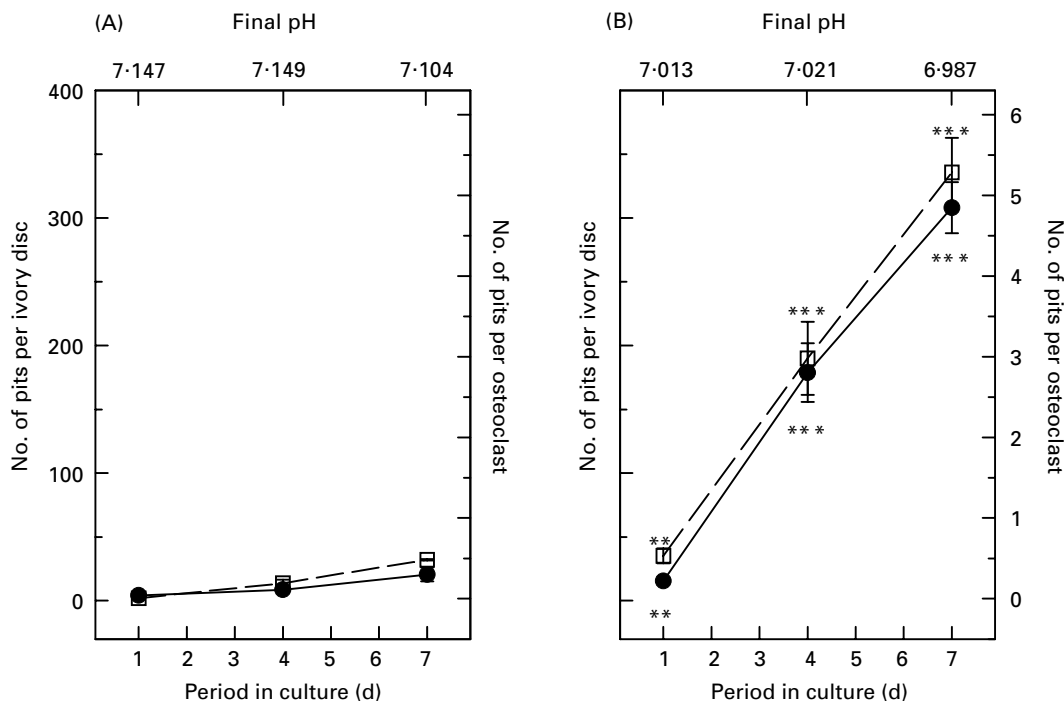
Fig. 6. Long-term stimulation of resorption pit formation by mature rat osteoclasts cultured on ivory discs in a slightly-acidified medium (medium + 6·5 mmol H$^+$; B) for up to 7 d. A 15-fold increase in resorption is associated with a mean pH difference of only 0·13 unit. (●–●), pits per ivory disc; (□— —□), pits per osteoclast. Values are means with their standard errors represented by vertical bars for five determinations. Significantly different from the respective values for the non-acidified control (A): **$P < 0·01$, ***$P < 0·001$.

such that a pH reduction of about 0·25 units is sufficient to cause a 9-fold increase in osteoclast-mediated Ca^{2+} release (Fig. 5). It was also found that the established osteolytic actions of agents such as parathyroid hormone, 1,25-dihydroxycholecalciferol and prostaglandin E$_2$ were themselves dependent on acidification (Fig. 8).

Mechanism of action of pH effects on bone cells

Several possibilities exist for the mechanism by which extracellular acidification activates and stimulates osteoclasts to resorb bone. Obviously, a low ambient pH will favour resorption simply by reducing the gradient against which osteoclasts must pump H$^+$ across the ruffled border. However, given the steepness of the response of the osteoclasts to small changes in extracellular pH, this factor seems unlikely to account for the entire effect. Thus, it is not unreasonable to imagine that a pH 'receptor' coupled to some signalling–amplification system could be present on or in the osteoclast. It is possible that a pH 'receptor' on the osteoclast cell membrane could be of the acid-sensing ion channel type described by Waldmann *et al*. (1999).

Although the mechanism by which osteoclasts detect pH changes is still unknown, progress has been made in understanding the 'downstream' responses of the key resorptive enzymes to extracellular acidification. Acidosis *in vitro* induces the activity of the vacuolar-type H$^+$-ATPase in osteoclast membranes within minutes (Nordström *et al*. 1997); this enzyme is thought to be primarily responsible for pumping H$^+$ out of the osteoclast into the extracellular

resorption compartment in order to dissolve hydroxyapatite. Recent work has demonstrated that upregulation of osteoclastic expression of mRNA for carbonic anhydrase II and for the calcitonin receptor occurs within 4 h after acidification (Biskobing & Fan, 2000). Carbonic anhydrase II is thought to play an important role in the intracellular generation of H$^+$ for resorption, and the calcitonin receptor is a marker for terminal differentiation of the osteoclast phenotype. Our own recent findings (A Brandao-Burch, S Meghji and T Arnett, unpublished results) show that bone organ cultures maintained at pH 7·0 exhibit striking up regulation of mRNA for tartrate-resistant acid phosphatase and cathepsin K (Fig. 11).

Dietary considerations

Metabolic oxidation of proteins containing S and P ultimately yields H$^+$ residues corresponding to H$_2$SO$_4$ and H$_3$PO$_4$, which must be excreted via the kidneys. The average US diet has been estimated to generate an inorganic H$^+$ residue of about 0·1 mol/d (Barzel, 1995), which is, for example, equivalent to approximately 8 ml concentrated HCl. Although high protein intake (particularly of animal protein) has often been regarded as a risk factor for low bone mass, strong data also exist (e.g. from the Framingham Study) for the opposite effect. It should also be borne in mind that vegetable proteins may deliver equivalent amounts of S (see Heaney, 2001). Green vegetables of the cabbage family also contain appreciable quantities of S. There is reasonable evidence that increasing
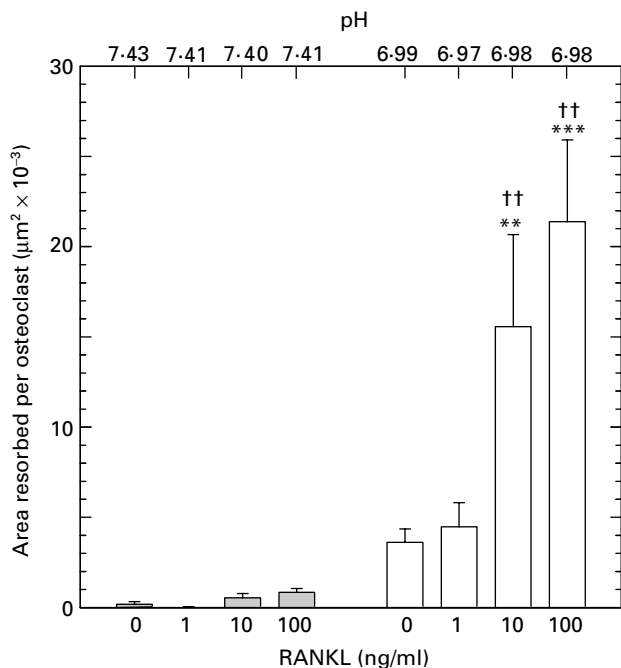
Fig. 7. Synergistic stimulatory effects of low pH and receptor activator of nuclear factor kappa B ligand (RANKL) on osteoclastic resorption. At physiological (blood) pH of approximately 7·4 (▨) basal resorption was very low and RANKL treatment caused small increases only. Combined treatment with RANKL (10 and 100 ng/ml) and low pH (☐) resulted in dramatic increases in resorption. Values are means with their standard errors represented by vertical bars for five determinations. Significantly different from values for the same RANKL concentration at pH 7·4: **$P < 0.01$, ***$P < 0.001$. Significantly different from the control values in the same pH group: ††$P < 0.01$.

Fig. 8. Dependence of the osteolytic action of prostaglandin E_2 (PGE_2), 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) and [1-34] bovine parathyroid hormone (PTH) on ambient acidification in 3 d cultures of mouse half-calvaria. Addition of 15 meq/l OH^- as NaOH (▨) increased final medium pH from approximately 7·1 in control cultures (☐) to approximately 7·3, resulting in marked attenuation of Ca^{2+} release in all treatment groups. Values are means with their standard errors represented by vertical bars for five determinations. Significantly different from the control group (C) values: * $P < 0.05$; *** $P < 0.001$. Significantly different from respective, non-alkalinized treatment group: † $P < 0.05$ (PTH), †† $P < 0.01$ (PGE_2).

dietary protein intake in human subjects and rats results in increased urinary Ca^{2+} and H^+ excretion (Trilok & Draper, 1989a,b). Another study with human volunteers has shown that increasing the dietary acid load without altering overall protein intake results in increases in urinary Ca^{2+} and collagen C-telopeptide excretion, suggesting increased bone resorption (Buclin *et al.* 2001). However, the influence of dietary protein on acid–base balance in blood is less clear. This area is not well studied, but available data suggest that omnivores may, in fact, have slightly higher blood pH than age-matched vegetarians with a lower protein intake, despite (or because of) slightly more acidic urine (Ball & Maughan, 1997). Further investigation into the long-term effects not only of protein, but also alkali-forming fruits and vegetables on acid–base status, bone mass and fracture risk would seem to be warranted (New, 2002; New *et al.* 2000).

Cola drinks may make a considerable contribution to dietary acid intake in some individuals. H_3PO_4 is added to such drinks to yield a pH of about 2·6; simple titration against NaOH shows that 1 litre of the common cola drinks contains an acid load equal to about 36 ml HCl (1 M), corresponding to about 40 % of the fixed H^+ generated daily by the normal US diet. For comparison, this amount of acid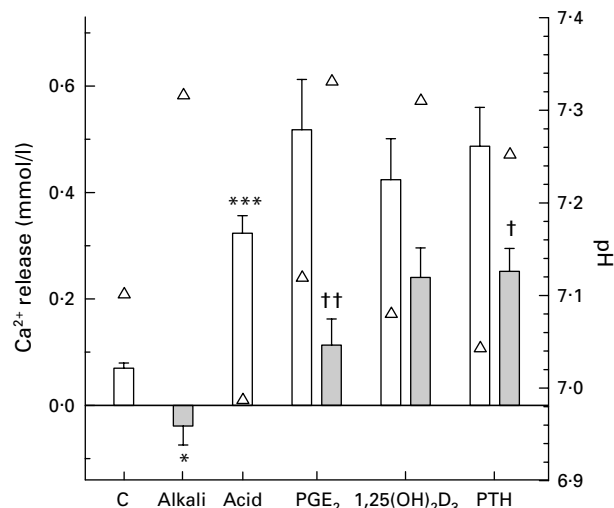 would be neutralised by approximately four 500 mg $CaCO_3$ antacid tablets (T. Arnett, unpublished results). However, any deleterious effect of the acid load from H_3PO_4 in cola drinks may be offset, at least for bone, because PO_4^{3-} is a powerful reversible inhibitor of the formation and activity of osteoclasts. It is also noteworthy that blood PO_4^{3-}, unlike Ca^{2+} levels, are not tightly regulated and may fluctuate markedly in normal mammals within the range that also regulates osteoclast function (about 2–4 mM; Yates *et al.* 1991).

Ageing and role of vasculature

With advancing age, there is a slight but notable decrease in blood pH and HCO_3^-, i.e. a progressive slight metabolic acidosis. This acidosis, which is probably of dietary origin, is ultimately due to the normal age-related decline in renal function (Frasetto & Sebastian, 1996; Frassetto *et al.* 1996). The general quality of the vascular supply around the body also tends to decline with age. In bone, available data indicate that ageing results in a progressive loss of the medullary blood supply, which is only partly compensated by an increase in the periosteal blood supply, leading to marrow ischaemia (Bridgeman & Brookes, 1996). This trend is also evidenced by the increase in yellow fatty marrow (at the expense of red marrow) with age. The consequence of such ischaemia is hypoxia, which acts as a powerful stimulus for osteoclast formation from marrow precursors (Gibbons *et al.* 2001), as well as causing a local acidosis, which will favour the resorptive activity of mature
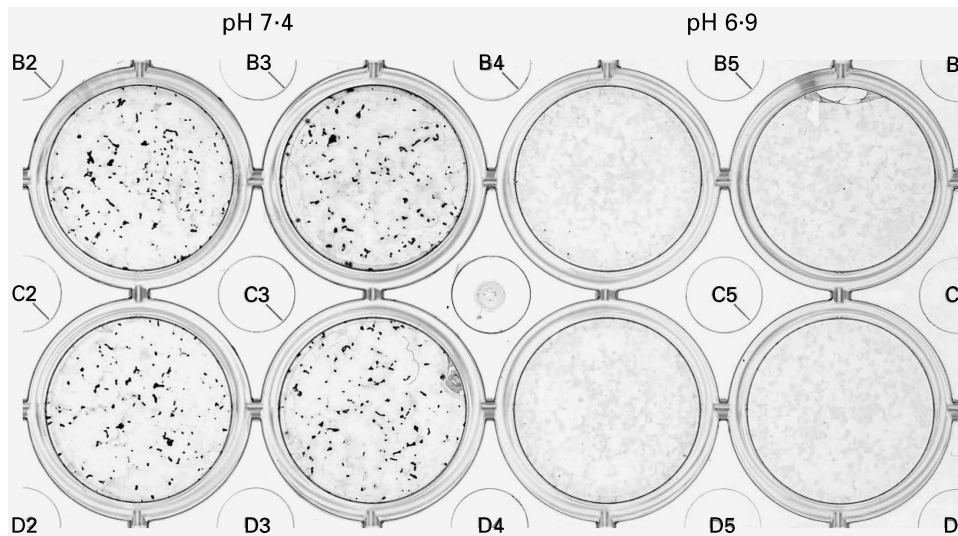
Fig. 9. Inhibition of bone nodule formation by acidosis. Primary rat osteoblasts were cultured in plastic wells for 14 d in control medium at pH 7·4 or in acidified medium at pH 6·9. Bony nodules consisting of mineralised extracellular matrix, visualised by alizarin red staining are evident only in control wells. (Reproduced by courtesy of Ms A. Brandao-Burch.)
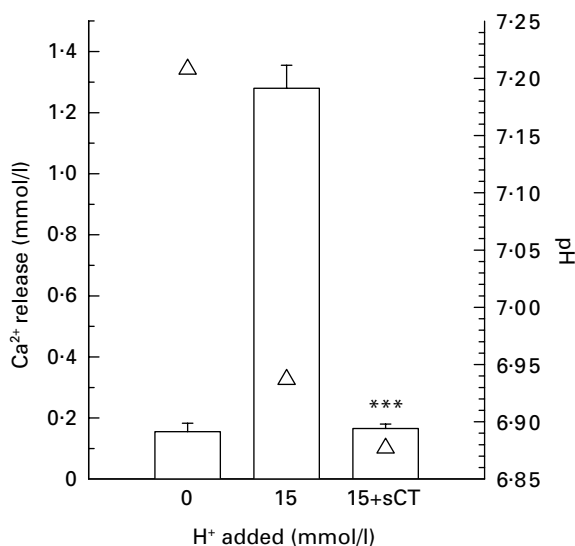

Fig. 10. Complete inhibition of HCO_3^- acidosis-stimulated Ca^{2+} release (□) from 3 d cultures of mouse half-calvaria by salmon calcitonin (sCT; 20 ng/ml). (△), pH. Values are means with their standard errors represented by vertical bars for five determinations. Significantly different from the control values: ***$P < 0.001$.

Fig. 11. Semi-quantitative polymerase chain reaction showing up regulation of mRNA for tartrate-resistant acid phosphatase (A) and cathepsin K (B) in mouse calvarial bones cultured at pH 7·05 (acid) for 3 d. No expression of cathepsin K mRNA was detected in controls or acid + receptor activator of nuclear factor kappa B (RANK) (pH 7·20). PGE_2, prostaglandin E_2. Values are means with their standard errors represented by vertical bars for three determinations. (Reproduced by courtesy of Ms A. Brandao-Burch.)

osteoclasts. Thus, maintenance of a healthy blood supply is likely to be of key importance to skeletal health.

Acid–base balance and anti-osteoporosis therapies

There is evidence that therapies aimed at limiting osteoporotic bone loss could exert some effects via alterations in systemic acid–base balance. The study of Orr-Walker *et al.* (1999) reported that hormone-replacement therapy with
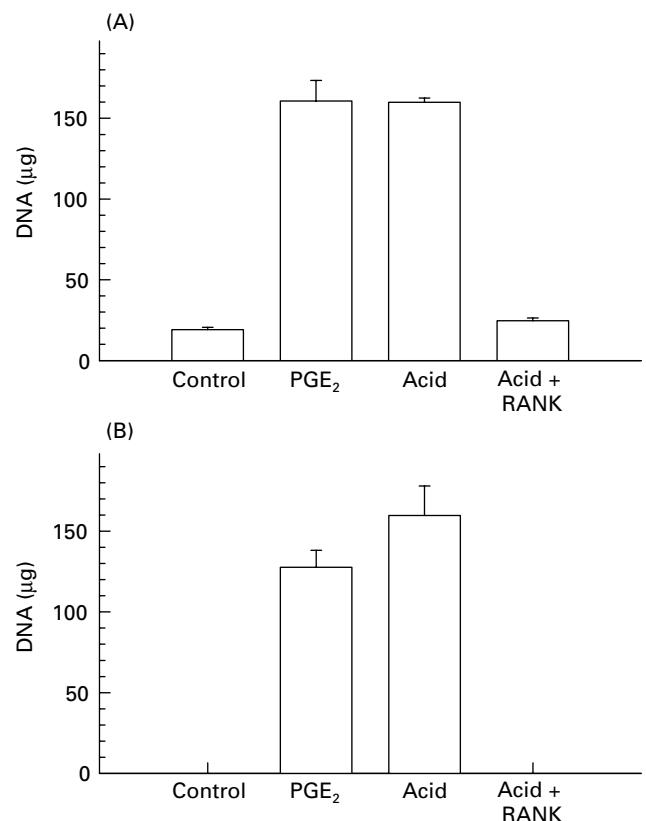
oestrogen and progestin causes a respiratory alkalosis in normal post-menopausal women, and that changes in blood pH were inversely correlated with those in urinary excretion of hydroxyproline, an index of bone resorption. The effects appeared to be due to a stimulation of ventilation by progestin. In the rat, testosterone deficiency due to orchiectomy results in mild metabolic acidosis and osteoporosis, which is alleviated by supplementation with alkaline salts (Straub *et al*. 2001). In post-menopausal women, dietary supplementation with $KHCO_3$ caused marked improvements in mineral balance and indices of resorption, as well as small increases in blood pH (0·02 units) and HCO_3^- (1·8 mmol/l; Sebastian *et al*. 1994). The bone-sparing effect of dietary supplementation with alkaline calcium salts is now well established, particularly for elderly women (Dawson-Hughes *et al*. 1990; Reid *et al*. 1995). Blood Ca^{2+} levels, which are tightly regulated in normal subjects, are not substantially altered by ingestion of large quantities of calcium salts. Whether a component of the beneficial action of calcium salts is simply a result of their alkaline nature remains to be determined. However, this idea is certainly consistent with the observations that the anti-osteoporotic effect of Ca^{2+} supplementation is due to an inhibition of osteoclastic bone resorption (Ginty *et al*. 1998; Scopacasa *et al*. 2000), and that alkaline calcium salts are most effective in the individuals who are likely to be acidotic, i.e. the elderly (Frassetto & Sebastian, 1996; Frassetto *et al*. 1996).

Conclusion

Cells are sensitive to external pH and their function is normally inhibited by acidic conditions. For this reason systemic acid–base balance must be maintained within narrow limits. Osteoclasts, however, are extremely unusual in that their activity is stimulated by, and is dependent on, low pH. This remarkable effect may represent a primitive 'fail-safe' mechanism that evolved with terrestrial vertebrates to correct systemic acidosis by ensuring release of alkaline bone mineral when the lungs and kidneys are unable to remove sufficient H^+ equivalent. The available evidence suggests that release of bone mineral in response to chronic acidosis is almost entirely an osteoclast-mediated process. Thus, the earlier concept that the skeleton functions as a passive 'ion-exchange column' with regard to acid–base balance must now be revised; the 'last defence' of systemic pH is perhaps too important to be left to physico-chemical processes. Given the great sensitivity of osteoclasts to ambient pH, it seems likely that even slight chronic acidosis could be sufficient to cause appreciable bone loss over time. Conversely, dietary or hormonal manipulations that result in a small extent of alkalosis might be expected to have a bone-sparing effect.

Acknowledgement

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