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Perspective Article

Hylonome

Calcium homeostasis: How bone solubility relates to all aspects of bone physiology

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Abstract

This perspective challenges the bone research community to study a new concept of calcium homeostasis and determine how it affects all aspects of bone physiology and disease. The concept started with Neuman's discovery that the apparent supersaturation of calcium in the extracellular fluid (ECF) could be explained by the presence of non-collagenous proteins on the surfaces of bone. His discovery opens the door to a new field of bone research and raises the question of how his result affects other aspects of bone physiology and pathology? The purpose of this perspective is to challenge the bone field to determine the significance of these findings. The report lists a few areas that need inquiry and supplies premises that need to be tested.

Keywords: ECF, Extracellular Fluid, PTH, Parathyroid Hormone

Introduction

Two recently published articles[1,2] reviewed the evidence that the non-collagenous proteins on bone surfaces determine the free calcium ion levels in the extracellular fluid (ECF). The basis of the most recent of these articles was the report by Neuman et al.[3,4] that a mixture of the non-collagenous protein of bone and bone mineral in a buffered solution increased the solubility of the bone mineral.

These are very significant observations that will require extensive studies to determine the role of bone solubility in all aspects of health and disease. There are many questions needing to be resolved by laboratory and clinical studies. This perspective is being written to list some of these areas of inquiry and some of the relevant questions. It is our hope that this will stimulate interest in these findings and the research that is necessary to answer the questions.

There are two important properties of calcium that require all vertebrates to control its free ion concentration in body fluids. These are the ability of calcium to bind to proteins, and the very low solubility of calcium phosphates. The following report assumes that the apparent supersaturation of ionic calcium in the ECF is explained by the presence of non-collagenous proteins on bone surfaces. Figure l is a diagrammatic representation of a cross-section of a Haversian canal in compact bone showing the relationship of bone surfaces to lining cells and osteocytes; to bone fluid spaces in contact with bone mineral; and to blood vessels supplying the area. The cells are enlarged to better visualize ECF spaces. Non-collagenous proteins are located in all areas where the bone fluid is in contact with bone mineral.

Two equilibrium processes

The binding of calcium to proteins and the solubility of its phosphate salts are both reversible processes and therefore involve an equilibrium phase. For this report, the term "equilibrium process" is used to refer to the fact that free ions in solution (in this case calcium and/or phosphate) are in a reversible reaction with a fixed phase in which the ions are attached to an inorganic or organic component. In these reactions, the specified ions are flowing continuously in both directions. Equilibrium is reached when the inward and outward flows of these ions are equal.

The first equilibrium that is important in calcium homeostasis is the binding of calcium to proteins. There are three

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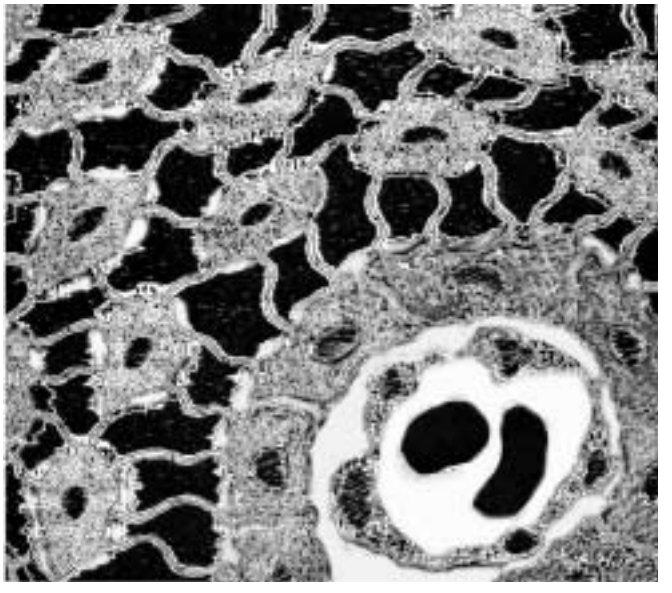

Figure 1. A diagrammatic presentation of a cross-section of a Haversian canal. The cells are several times actual size. Mature bone is in black. Non-collagenous proteins are located on all bone surfaces bordering fluid spaces. These are between the lining cells and bone mineral; around osteocytes and between the protoplasmic extensions and the bone. Reprinted with the kind permission of Springer Science and Business Media[27].

relevant situations where calcium ions in the fluid come in contact with proteins. The first is in blood plasma. When the process is at equilibrium one half of the calcium in plasma is attached to protein and is continuously exchanging with the free calcium. This is important only because it illustrates the speed at which the calcium ion equilibrates[3,5]. The next situation is the relationship of free calcium in the ECF to proteins contained in cell walls. Proteins determine the strength of the membrane and influence the passage of ions and compounds into the cell. Also it is important with nerve and muscle cells because the calcium level determines their excitability. This reaction makes calcium ion control a necessity. The third situation is the equilibrium between the calcium in the ECF and that on the non-collagenous proteins on the bone mineral surfaces. There is a continuous two-way movement of calcium ions between the fluid and the proteins. In an earlier paper[1], Talmage proposed that this process at bone surfaces was an integral part of calcium ion control.

The second type of equilibrium that is the basis for calcium homeostasis is the solubility of hydroxyapatite. Solubility is an equilibrium in which the ions in question are continuously entering and leaving the solid phase even when the two movements known as influx and efflux are equal. This movement of calcium on and off the bone occurs whether the solubility of bone mineral is very low or is increased by its contact with noncollagenous proteins. This is a very important concept, which is illustrated by the action of parathyroid hormone (PTH). Parathyroid hormone is usually considered to act by increasing the efflux of calcium from bone mineral by stimulating the activity of osteoclasts. However, the release of calcium from bone cannot raise the concentration of calcium in the ECF unless there is also a change in the equilibrium level set by the reaction of bone surface proteins.

In conclusion, any study of calcium ion control must involve the two equilibration processes discussed above, as they are both the cause of and the solution to the need for calcium control. This is a fruitful area for research.

The lining cell–osteocyte complex

All surfaces of bone mineral in contact with the ECF are covered with a single layer of lining cells[6,7]. These cells have protoplasmic extensions that extend into the bone matrix and are connected with osteocytes that are surrounded by bone mineral. The lining cells are separated from neighboring cells by open channels but are connected by gap junctions (Figure 1). Each lining cell was at one time an osteoblast that was also connected to the matrix by protoplasmic extensions[7]. Therefore, the lining cell is fixed in place by these extensions and by the gap junctions. However, there is space between the lining cell and the bone mineral, and the cell has some movement in its allotted space. The non-collagenous proteins are located between the cells and the bone surface and do not penetrate the channels between cells[8].

A controversy still exists as to whether the blanket of lining cells form a membrane that separates the bone fluid at the surface of bone from the rest of the ECF[5]. Neuman and his colleagues postulated the existence of such a membrane but after much study rejected the idea as impossible[9]. Matthews and his colleagues were among those who showed by electron microscopy that the channels between lining cells were open[10]. Figure 2 is an electron micrograph from a rat tibia. This animal was injected with lanthanum just prior to sacrifice. Notice that the stain fills the open channels between lining cells and is mixing with the bone fluid. It will even reach the fluid in osteocyte lacunae. Bronner concluded that if the membrane existed, the cells would have to pump large amounts of calcium ions intracellularly and that this was energetically impossible[11]. Grubb et al. studied the rapid entry and return of ^{45}Ca from bone during PTH or calcitonin infusion[12,13]. Finally, Neuman's demonstration that non-collagenous proteins on the bone surface increase the solubility of bone mineral[3,4] removes the original reason for proposing a membrane. However, the lining cells while not inhibiting diffusion by their location may increase the time required for the situation at bone surfaces to equilibrate with the general ECF.

A question remains as to whether lining cells can synthesize proteins. Each lining cell is derived from an osteoblast. This latter cell is a proven factory for synthesizing large amounts of collagen and is the major source for non-col-

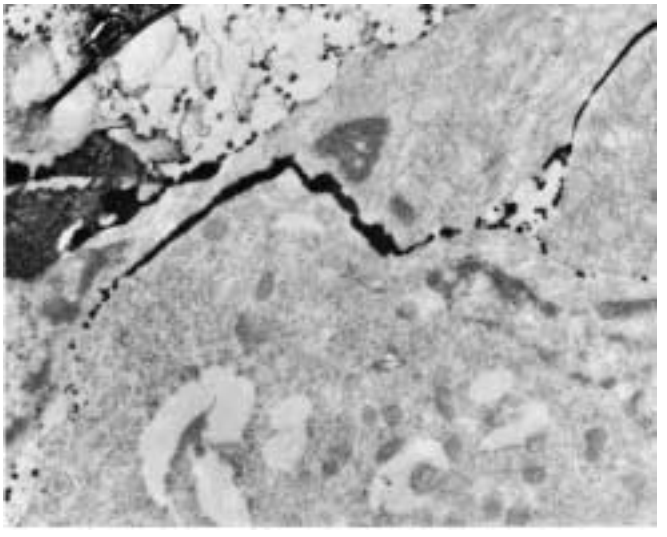

Figure 2. An electron micrograph of lining cells bordering bone surfaces. The rat was injected with lanthanum prior to sacrifice. The lanthanum stain, appearing black, has already filled the channels between lining cells and is mixing with the fluid bordering bone mineral. Reprinted with the kind permission of Springer Science and Business Media, New York[28].

lagenous proteins[14]. Anatomists have described lining cells as quiescent cells, suggesting that when an osteoblast became a lining cell it ceased making protein. What is the source of the non-collagenous proteins found under the lining cells? Once formed do they remain functional indefinitely or do they need to be replaced regularly by the lining cell-osteocyte complex? The lining cells must be considered active, as they are the primary target cells of both parathyroid hormone and calcitonin.

It is probable that the microenvironment of lining cells is modified by the size, type and location of nearby blood vessels. There may be different diffusion patterns in various locations in bone. Thus, there may be areas in bone where there is a net movement of calcium out of bone while in other sections the net movement may be zero or an influx into bone. Such a situation might explain why bone density increases in areas where muscular activity is high. This problem was illustrated in a recent report by Sievanen in an article entitled "Hormonal influences on the muscle–bone feedback system"[15].

The conclusion drawn from this and the previous section is that bone mineral surfaces are areas of very high activity instead of quiescent areas as they have been labeled. In addition, the lining cell–osteocyte complex makes up by far the greatest portion of cells in bone. We suggest that much more calcium enters and leaves bone through these equilibrium processes than by the process of bone remodeling. This is an area in our report that might incur some disagreement and therefore merits much further study.

Parathyroid hormone, calcitonin and phosphate

The influence of parathyroid hormone on renal phosphate excretion was the first action of PTH that was confirmed. This occurred in the early 1900s[16] and by the late 1940s was a widely accepted basis for PTH action[17]. Unfortunately, it was based on the assumption that plasma was saturated with CaHPO4. The amount of calcium in the plasma, therefore, was thought to be dependent upon the product of Ca^{2+} and $HPO4^{2-}$. If the kidney increased its excretion of phosphate, this would permit an increase in plasma calcium. The fact that plasma was not saturated with CaHPO4 and that nephrectomized animals responded normally to PTH negated this as the method by which PTH controlled plasma calcium. However, the hormone's ability to increase renal phosphate excretion is still a valid action of the hormone. Since phosphate is in excessive supply in mammals, the kidney threshold for its excretion appears to be the primary control of the level of phosphate maintained in the ECF.

The observation that both PTH and calcitonin produce changes in lining cells has been well confirmed[7,10,18-20]. However, it is not known how these effects relate to a change in the solubility of bone mineral. The classical explanation of the rise or fall, but in opposite directions, of plasma calcium levels produced by these two hormones is that it was caused by their effects on the bone resorptive processes. This must now be attributed to changes in the solubility of bone mineral produced by the non-collagenous proteins. In Neuman's experiments in which he mixed the non-collagenous proteins with bone mineral, the solubility of the bone was dependent on the ratio of protein to mineral. However, the suggestion that PTH induces and calcitonin decreases the production of the relevant proteins seems unlikely because of the rapidity of the hormone effects. Another possibility is that PTH and calcitonin have opposite effects on the production of an accessory molecule that either enhances or reduces the activity of that protein. Neuman found that one of the non-collagenous proteins was phosphorylated. Also, a large number of other experiments suggest that phosphate may be involved in calcium homeostasis in some way that allows it to affect the bone surface control of calcium homeostasis[20-22].

Although the last hypothesis is speculative, it is supported by some interesting observations. The first is that the parathyroid glands appeared in vertebrates when the supply of phosphate shifted from limited to excess, occurring when vertebrates began to appear on land. Also, Copp et al.[23] reported that if rats were maintained on a very limited phosphate diet, the parathyroid glands tended to atrophy and some times disappear. Recently, these findings were extended by Jara et al.[24], who reported that when rats were kept on a phosphate-free diet there was a gradual rise in the plasma calcium levels and a decreased effectiveness of injected PTH. The injection of a higher than physiological dose of calcitonin was followed by the appearance of a precipitate of $CaHPO_4$ around the lining cells and osteocytes. Numerous studies have demonstrated that the responses of calcium and

phosphate to calcitonin were different. For example, they have shown that phosphate was affected by calcitonin first, followed by an effect on calcium.

As more information is obtained concerning the mechanism of action of the non-collagenous proteins in increasing the solubility of bone mineral, the role of phosphate in this process should become clarified. This is an area where additional research is needed.

Finally, we would like to suggest that the secretion of calcitonin is controlled by food passing through the digestive tract. This aspect received considerable attention years ago[25] but was abandoned because of irregularities between different animals used. Study in this area should be resumed.

The effect of renal excretion of calcium and phosphate on maintenance of free calcium levels in the extra cellular fluid

In this report we have elaborated on the premise that the control of the free calcium level in body fluids is determined by the degree of solubility set by the relation of non-collagenous proteins to bone mineral. This is an equilibrium process with continuous two-way movement of calcium and phosphate into and out of bone mineral. At equilibrium, the influx and efflux are equal; but under certain circumstances, they are unequal and there can be a net movement of calcium in one of the two directions. In this case, there will be a slow loss or gain of the calcium in bone mineral. The free ion concentration in the ECF can only be changed by changing the equilibrium level at bone surfaces.

How does the renal excretion of calcium and phosphate affect this control system? Many studies have proven that nephrectomized animals can maintain the expected ECF calcium levels whether PTH is present or not[26]. While this is true, under normal physiological conditions the renal function must impinge on the basic process at the surfaces of bone. The kidneys set a threshold level above which the excretion of calcium increases. This process ensures that the concentration of a specific ion does not become dangerously elevated. The renal system can prevent an elevated concentration of an ion, but it cannot prevent it from falling below needed levels when the external source becomes limited. In all vertebrates except elasmobranches, the solubility level set at bone surfaces uses the bone mineral to prevent such a decrease in the plasma level of the calcium ion.

A problem arises when the kidney threshold for calcium loses its co-ordination with the solubility level set at the bone surface. For example, PTH raises the free calcium level in the ECF by adjusting the solubility of bone mineral. Under normal conditions, the renal threshold rises to match this ECF level. If the renal threshold is set lower than that at bone surfaces, and the individual is on a low calcium intake, there could be a continuous withdrawal of calcium from bone mineral. If the kidney threshold is set higher than that at bone surfaces and the calcium intake is normal, there could be a continuous net movement of calcium into bone surfaces. Normally the kidney and bone work together, but a discrepancy could occur both in the presence and absence of PTH. Is it possible that this could be the cause of the genetic disease in which bone continues increasing density throughout life? Or could the opposite occur, in which there is a continuous bone loss over time. This problem should be of interest to all clinicians who deal with metabolic bone diseases.

Summary and conclusions

This report is full of challenges–challenges to thoroughly study this new concept of calcium homeostasis. The concept started with Neuman's discovery that the apparent supersaturation of calcium in the ECF could be explained by the presence of non-collagenous proteins on the surfaces of bone. The delayed response to his discovery raises the question of how his result affects other aspects of bone physiology and pathology. The purpose of this perspective is to challenge the bone field to determine the significance of these findings. The report lists a few areas that need inquiry and supplies premises that need to be challenged. A few of these are listed as follows:

1. Can the chemical studies of Bill Neuman be repeated and extended? Using his *in vitro* model, must the non-collagenous proteins be added continually to the bone mineral to maintain solubility or can the proteins act indefinitely without replacement of cells, protein or energy?
2. How important are phosphate ions in the overall control of calcium homeostasis? Do the hormones, calcitonin and PTH, utilize phosphate to exert their influence on calcium ion concentration?
3. Can the premise be challenged that the exchange of calcium and phosphate is very rapid at all bone surfaces and that this activity is important in many aspects of bone physiology and pathology?
4. Can studies in marine forms, including teleosts, elasmobranches and mammals, help us understand calcium control in humans?
5. And last but not least, accepting the premise that the solubility of bone mineral is increased by its relationship to non-collagenous protein, how does this correlate with the well-known renal processes for calcium and phosphate excretion? Can calcium be removed or added to bone rapidly by adjusting renal excretion?

Bone research is very important as well as the study of its pathology. This report will only be a success, if these are stimulated.

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