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Calcium homeostasis and bone surface proteins, a postulated vital process for plasma calcium control

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Abstract

This report is a more in-depth explanation of a recently reported hypothesis[1] for controlling the ionic calcium content of plasma and extracellular fluids (ECF). The hypothesis proposes a two-step process for returning calcium to the ECF against the established gradient continuously moving calcium from plasma to bone surfaces. The first step in this process is the predicted transfer of calcium directly from bone surfaces to the non-collagenous proteins, which are in contact with bone mineral. This calcium would be complexed to existing proteins and a portion would automatically become available for equilibration with ionic calcium in the ECF. The basis of the hypothesis is that the equilibration level helps to set the ionic calcium concentration of plasma. The gradient toward bone and the proposed two-step return occur in the ECF of bone and would be considered normal physiochemical processes. Thus, these processes are critical for mineral ion homeostasis in mammals. In this hypothesis, parathyroid hormone (PTH) is not required for the basic process. However, PTH works within the process to raise and set a precise plasma calcium concentration. The report to follow describes the process and discusses its relationship to normal and pathological conditions affecting human health.

Keywords: Calcium Homeostasis, Bone, Proteins, Parathyroid Hormone, Plasma Calcium

Introduction

One of the characteristics that sets all vertebrates apart from other animal life is the presence of a living, bony endoskeleton. This skeleton is well developed in land vertebrates who depend on it for structural support to permit motion and locomotion. The inorganic material component is primarily a crystalline form of tertiary calcium phosphate $[Ca_3(PO_4)_2]$, with minor contributions from other low solubility calcium salts. One of the requirements for maintaining a living endoskeleton is an adequate blood supply to furnish oxygen and nutrients to the incorporated cells. The net result is that the circulating extracellular fluids (ECF) have rapid access to all portions of the skeleton and are in continuous contact with not only the cells but with the insoluble calcium phosphate substrate.

One of the critical functions of calcium in the body of vertebrates is in the maintenance of the structure and integrity of cell membranes. This is accomplished by the complexing of calcium ions to the protein molecules whereby both the conformation and function of the protein may be affected. For most proteins in the body, this process is reversible. Under normal homeostatic conditions, the amount of calcium complexed is partly determined by the ionic concentration of the medium with which it is in contact. Therefore, it could be concluded that the ionic calcium concentration of the medium (such as the ECF) must be kept at an appropriate level for proper maintenance of cell membrane integrity. Intracellular calcium levels are also maintained within narrow limits and affect a myriad of cell functions. With the ECF buffered at a pH of 7.4, phosphate, also an important constituent in ECF, is primarily in its divalent form (HPO_4). The calcium salt of this phosphate ($CaHPO_4$) is relatively soluble. Plasma, therefore, is able to contain an adequate amount of calcium needed to maintain the integrity of cell membranes. This amount of calcium is far above the solubility of the inorganic component of the living endoskeleton with which the ECF is in continuous contact. Consequently, on each passage of blood through bone, some calcium and

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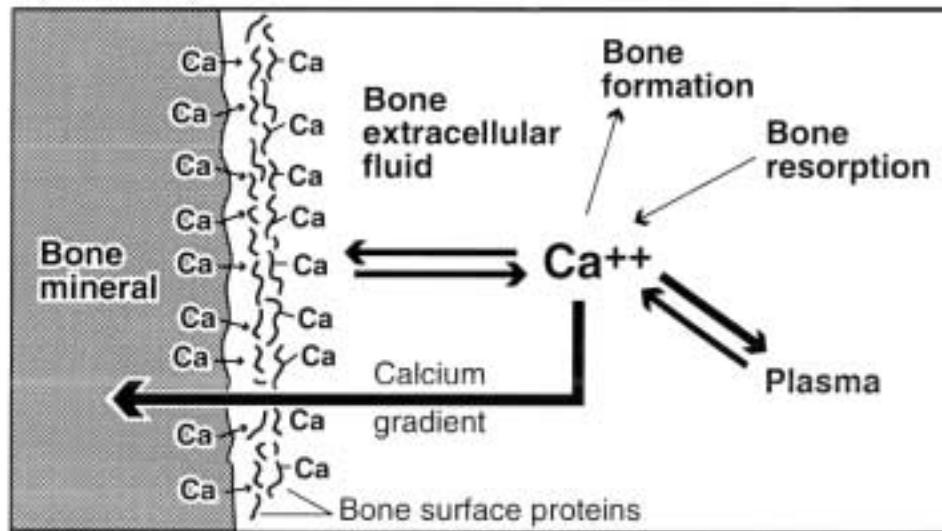*E-mail: lester1@mail.nih.gov*

Figure 1. A diagrammatic representation of the movement of calcium into and out of bone as described by our proposed hypothesis for calcium homeostasis. The location of the non-collagenous proteins is represented by the squiggle line fragments adjacent to the bone mineral surfaces. The thickness of the arrows represents relative differences in the volume of calcium moved.

phosphate would be routinely transferred from the ECF to bone mineral.

The described situation resulted in the development of another characteristic of vertebrates, particularly those living on land; a strong calcium gradient from plasma and the ECF into bone mineral. This gradient was studied extensively after World War II when radio-calcium became available as a research tool. Based on the work of many investigators, it is now generally accepted that this gradient, in the average 70 kg human, moves at least 6 g/day of calcium from blood to bone mineral. Thus, an amount of calcium equivalent to the entire plasma content of calcium is moved out of the ECF every few hours. It is obvious that if the vertebrate is to maintain the necessary plasma calcium content there must exist mechanisms to return sufficient calcium from bone to the ECF to match that continuously lost to bone as a result of the gradient. The purpose of this report is to present further details and to expand upon a proposed two-step process for accomplishing this task. This hypothesis was recently proposed in this journal[1]. The entire process of calcium movement from blood to bone and back to blood is illustrated in Figure l. The following section is an explanation of this sequence.

Explanation

Calcium movement into and out of bone

For land vertebrates the only external source of calcium is dietary calcium intake and calcium absorption via the digestive tract. Calcium enters the blood in its ionic form, where it equilibrates rapidly with calcium complexed to plasma proteins (Figure 2). Calcium bound to these proteins func-

tions in metabolic activities such as blood clotting; but for the purpose of the current discussions, we will focus on the rapid equilibration between calcium in its ionic form and that complexed to proteins. Approximately 50% of the calcium in the plasma is bound to proteins.

As blood flows through bone, the calcium it supplies to the local ECF is at the approximate ionic concentration found in plasma. Calcium ions react with all bone surfaces reached by the flowing ECF, thereby providing a constant calcium supply for the seeding and growth of hydroxyapatite crystals in areas of bone formation. However, in the bones of adult humans only a small percent of bone surfaces are required for bone formation activities. The fluid also reaches areas of osteoclastic bone resorption, which adds to the ECF calcium from the breakdown of bone. The area covered by the resorption process is even less than for bone formation. Thus, the entire process of bone remodeling in the mature human uses only a small percentage of the total bone surface. Most of the bone surfaces consist of hydroxyapatite crystals and their hydration layers.

On contact with the ECF medium, hydroxyapatite crystals can both absorb and release calcium and phosphate ions into the fluid. However, because the local ECF is supersaturated with these ions, the next movement of both calcium and phosphate is into the bone mineral. This process is the basis of the calcium gradient shown in Figure 1.

The next step is the critical phase of our new hypothesis. Essentially all bone surfaces appear to be coated with organic material[2]. Most of these organic compounds attached to bone mineral surfaces consist of various non-collagenous proteins, some of which are noted for their high capacity to bind calcium. The best known are osteocalcin and osteonectin;
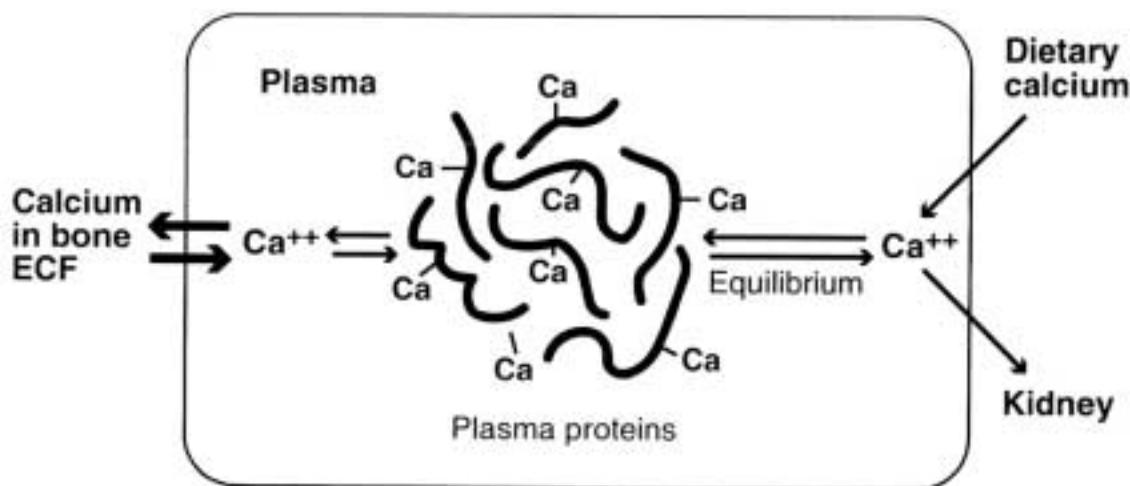
Figure 2. A diagrammatic representation to illustrate the rapid equilibration between ionic calcium and calcium in complex with proteins in plasma.

the latter is a phosphorylated glycoprotein. Both bind directly to hydroxyapatite crystals. Phosphoproteins extracted from bone also bind to hydroxyapatite[3]. We postulate that these and other calcium binding proteins are able to acquire calcium directly from bone mineral. We also propose that these are physiochemical reactions requiring little or no energy from an outside source. One possibility is that the transfer of calcium from mineral to protein occurs by exchange of two sodium ions attached to the protein for one calcium ion from the crystal lattice. This is supported by reports that bone surface proteins contain large amounts of sodium[4]. The second possibility is that proposed by the work of Hauschka[5]. He demonstrated that in combination with calcium, some bone surface proteins modify their conformations to a form that fits into the lattice of hydroxyapatite. Thus, the bound calcium matches calcium locations on the crystal lattice, which provides for the exchange of calcium from apatite to protein and vice versa. Either of these processes could maintain the bone surface proteins saturated with calcium.

The calcium complexed to these proteins would then react in the usual manner with the ionic calcium in the bathing medium by rapidly reaching an equilibrated state. The unusual aspect of the reaction is that, with a continuous bone mineral source for calcium atoms, the equilibration level is determined by the protein complexed calcium and not by the calcium ion concentration of the medium (which is determined by the concentration in plasma). Therefore, the amount of protein complexed calcium available to the ECF (we term this the exchangeable fraction) determines the ionic concentration of the ECF being returned by blood flow to the plasma. The entire process of calcium entry to and return from bone, which is the balance of three competing physiochemical reactions (Figure 1), sets the basic ionic concentration of calcium in blood.

The anatomy and geography of bone surfaces

Figure 3 presents a diagrammatic representation of the morphology of mature bone. The arrangement of the lining cells is important. Each cell is connected by gap junctions on protoplasmic extensions to osteoblasts or lining cells and to underlying osteocytes. The channels between these cells allow for free movement of extracellular fluid between cells and along the mineral surfaces. Not shown in the diagram are the locations of the non-collagenous protein molecules, which are found between the mineral and the lining cells and osteocytes. Thus, they are in contact both with the mineral and the bathing fluids. The cellular arrangement in bone is shown in two electron micrographs in Figures 4 and 5.

The phosphate ion and the calcium equilibration process

The extent to which phosphate aids or interferes with the entire postulated process for plasma calcium control is difficult to assess. Unfortunately, in most calcium research protocols, phosphate is usually considered as the 'tag along' ion and is often overlooked. The relationship of phosphate to our hypothesis can be divided into two categories: the first relates directly to maintaining phosphate homeostasis, and the second, to the effect of phosphate interaction with calcium homeostasis.

Abnormalities in phosphate homeostasis are not well understood. However, bone crystal growth requires both calcium and phosphate, so it is assumed that there is also a phosphate gradient toward bone. The control of plasma phosphate is primarily due to renal handling. A set plasma concentration is not critical in the physiology of the animal and the mechanism of the return of phosphate from bone has not been studied.

The second category involves the possible interaction of phosphate with calcium. When calcium is transferred from
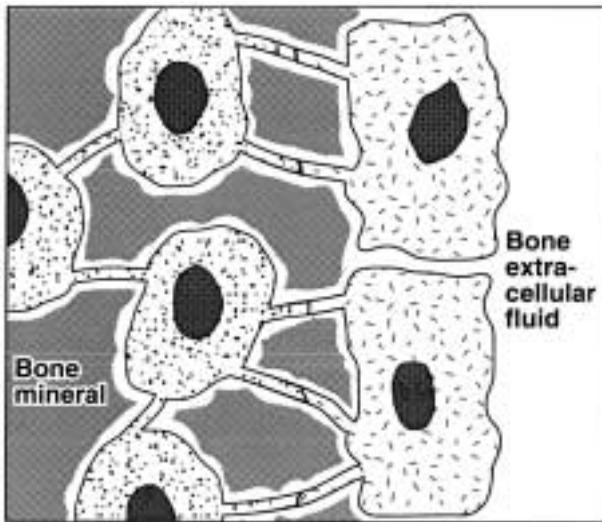
Figure 3. A diagrammatic representation of bone cells, mineral and extracellular fluid at surfaces of mature bone. Of particular importance are the channels between lining cells that allow for free movement of fluid. Not shown are the non-collagenous proteins that are located between the cells and the bone mineral surfaces.

Figure 4. Scanning electron micrograph of bone lining cells on surface of bone. Note the narrow open intercellular gaps between cells (X 1,000).

bone mineral to protein, some phosphate is released directly into bone ECF; this ion could react directly with the calcium equilibration process. If the ion reacts with bone lining proteins, it could play a role in the size of the exchangeable fraction of protein complexed calcium. While these are hypothetical projections, we believe that the study of calcium cannot be considered complete without coordination of the movements of both calcium and phosphate.
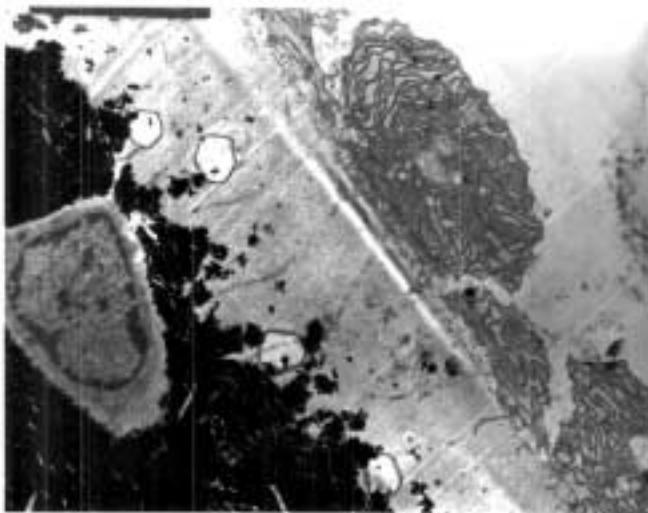

Figure 5. Transmission electron-micrograph showing bone lining cells on surface of bone contiguous with buried osteocytes; adjacent surfaces of mineralized bone are bathed by ECF as diagramed in Fig. 3 (X 6,500).
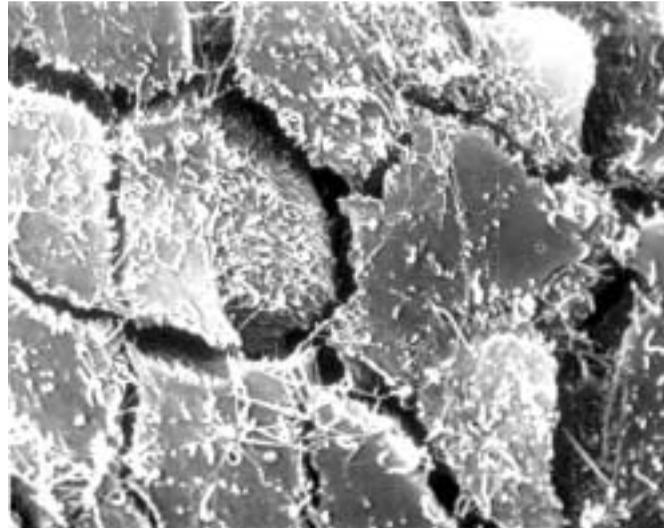
Parathyroid action in this equilibration process

As discussed above, one of the important premises of our hypothesis is that the size of the "exchangeable fraction" of calcium complexed to surface proteins helps to determine the equilibrated level of calcium ions in the bone ECF and, therefore, the calcium concentration of plasma. It follows from this premise that the only way parathyroid hormone would be able to raise plasma calcium concentrations above the basic concentration would be to increase the size of the 'exchangeable fraction'. There are two possible ways in which the hormone could increase the size of this compartment. One would be to affect the conformation of the surface proteins permitting more of the complexed calcium to become exchangeable. The second method might be that the hormone removes material that interferes with the equilibration process.

Parathyroid hormone does not directly affect the extracellular environment. The hormone achieves its effects by action on lining cells and their underlying osteocytes, all of which have membrane receptor sites for the hormone[6]. These bone cells are then stimulated to release a "messenger", which is able to act on surface proteins to increase the amount of exchangeable calcium. How this is accomplished can only be speculated. A possible candidate for the "messenger" secreted by the lining cells is a phosphatase, which would act on phosphate related to bone mineral and surface proteins. The importance of phosphate to parathyroid action is inferred by the report of Copp et al.[7] that demonstrated the atrophy and eventual disappearance of parathyroid glands in rats maintained for extended periods on a phosphate-free diet. Such a relationship between parathyroid hormone and phosphate was further supported by a recent study from Jara et al.[8] that demonstrated the gradual rise of plasma calcium and relative ineffectiveness of administered exogenous

parathyroid hormone in rats on a phosphate-deficient diet.

Phosphatases are commonly occurring enzymes in nature. Once released by the bone cells, such phosphatases could react with phosphate to set a higher equilibration level between the complexed and the ionic calcium in solution. Parathyroid hormone secretion has a tightly controlled negative feedback system based on the level of ionic calcium in plasma. The hormone would establish and maintain a controlled equilibration level between calcium complexed to bone surface proteins and the ionic calcium level of the ECF; the result of this action would be to establish and maintain a controlled and constant plasma calcium concentration. It is suggested that both the basic process of plasma calcium control and its ultimate adjustment by parathyroid hormone require only minimal expenditure of metabolic energy.

Vertebrate bone can continuously supply calcium to plasma in times of low calcium stress

Many years ago, Hastings and Huggins[9] demonstrated the rapidity in which plasma calcium returned to its prior level after dogs were subjected to an acute calcium loss. This occurred in both normal and parathyroidectomized animals. A short time later, Talmage and Elliott[10] extended this study in nephrectomized rats undergoing peritoneal lavage (this technique in rats substitutes for the process of dialysis in humans). The low calcium stress was provided by the use of a calcium-free rinse for the lavage. It was determined that, even in the absence of the parathyroids, rat bone was able to continuously supply calcium to the ECF at rates as high as 13 mg per kilo per hour. In the presence of functioning parathyroid glands this maximum rate was increased only to 16 mg per kilo per hour. When examined after 36 hours of continuous lavage, no gross histological changes could be seen in those bones removed from parathyroidectomized animals, while the usual increase in osteoclasts occurred in parathyroid intact rats. If these data were extrapolated to a 70 kg human, it would indicate that calcium could be removed from human bone at rates up to 1 gm per hour.

These studies are included in the presentation of our new hypothesis as they support the concept that the basic level of calcium return from bone is an extracellular physiochemical process capable of handling large quantities of calcium.

Relationship of the hypothesis to other factors of bone biochemistry

The purpose of this section is to relate our hypothesis for plasma calcium control to other known factors or processes affecting bone metabolism. There are two primary ways that our proposed process can be affected. One is by changing the volume of exchangeable calcium complexed to bone surface proteins by changing the conformation, the amount of the proteins, or by removing interfering substances. Any of these three mechanisms will alter the level of ionic calcium in the ECF. The other is to add or remove calcium from the system. Changes in the supply of calcium would not change the equilibration level, but would affect the time of equilibration and the proportional sources of calcium entering the system.

Dietary Calcium

Calcium from the intestine enters the ECF pool directly and immediately becomes a part of the gradient toward bone. If the amount is sufficient to raise the ECF ionic calcium concentration above the equilibration level for ionic and complexed calcium, it will cause a temporary disequilibrium and will enter into the process but will not change the equilibrated level. In humans, hypoparathyroidism (no matter what the cause) is managed by vitamin D and extra dietary calcium with plasma calcium maintained in the 8 mg/100 ml range. This suggests that a continuous input of dietary calcium is able to keep the system in a state of disequilibrium and appears to raise the plasma calcium level. However, on cessation of dietary calcium input, plasma calcium will decrease to the basic equilibration level.

Bone Resorption

Calcium entering the system from resorbed bone acts similarly to that entering from the diet except that it enters at a different point. Under normal physiological conditions the amount is too small to affect plasma calcium levels. However, any calcium entering the system does reduce the amount of calcium required from the supply of calcium released from surface proteins. In cases of severe hyperparathyroidism, the hormone increases the equilibration level directly, and at the same time adds considerable calcium to the system by its effect on osteoclastic bone resorption. Both processes stimulated by the excessive parathyroid hormone are occurring at the same time, placing the system in disequilibrium. Under these conditions, both mechanisms combine to produce an elevated plasma calcium concentration.

It should be kept in mind that, under normal physiological conditions, the level of circulating parathyroid hormone is dependent upon its need to maintain the level of plasma calcium. Therefore, factors affecting this need, such as plasma phosphate levels, will determine the degree of PTH effect on the bone remodeling system. Thus, phosphate is a major factor in establishing the hormone as a bone anabolic or catabolic agent.

Other hormones that might interact with the proposed process

In this category we would include the classical calcium regulating hormones: vitamin D and calcitonin. Vitamin D was mentioned above and will be discussed later. There are significant questions concerning the physiological significance of calcitonin[11]. We must wait for clarification before confusing the issue by any attempt to relate its action to our proposed hypothesis.

The one hormone (or group of hormones) that appears to

directly affect the equilibration process is cortisol. Our interpretation of two previous studies[12,13] is that that this hormone raises slightly the basic equilibration. Since only the basic plasma calcium concentration is affected, parathyroid hormone would hide such an effect by altering its rate of secretion. We postulate that the action of cortisol may be on the structure, conformation or quantity of bone lining proteins. Such changes could alter the amount of calcium complexed to these proteins or the amount available for exchange. We further suggest that this is not a bone specific action of the hormone but a part of its generalized action on all cells.

The relationship of the hypothesis to metabolic bone diseases

We include in this section brief consideration of pathological conditions resulting from calcium deficiency and bone remodeling disorders. Our hypothesis considers calcium homeostasis and bone remodeling to be two separate systems. Thus, as long as there are sufficient areas of mature hydroxyapatite with attached bone surface proteins and covered by a layer of lining cells, the process of calcium homeostasis will prevail and plasma calcium concentrations will be normal.

Osteoporosis is a condition in which there is a gradual loss of bone due to increased resorption and/or decrease in bone formation. The bone appears to be normal and calcium homeostasis is rarely affected. Osteopetrosis is a condition in which there is a lack of remodeling processes usually accompanied by the absence of osteoclasts. The bone eventually becomes abnormal due to the lack of repair. However, until bone surfaces no longer fulfill the requirement to permit calcium control, plasma calcium concentrations will remain normal. These two conditions are essentially remodeling disorders.

In vitamin D deficiency syndromes, the bone deficiency is in the rate and amount of mature hydroxyapatite formed. However, plasma calcium equilibrium levels can be normal until such times as calcium deficiency becomes acute due to malabsorption or when plasma phosphate levels are elevated. Thus, vitamin D deficiency is a potential homeostatic disorder. Only a few bone pathological conditions have been covered. If our hypothesis is correct, plasma calcium control should remain normal until there is a major loss of bone surfaces fulfilling the requirements needed for ionic calcium control. In most instances, the pathology is corrected or terminated before this occurs.

Conclusions

In this report, we have elaborated and expanded upon a new two-step hypothesis for the return of calcium from bone to blood to compensate for the opposing gradient moving calcium from plasma onto bone surfaces. The first step in this process is the transfer of calcium directly from hydroxyapatite crystal to the adjacent proteins that cover the mineral surface. As noted early in this paper, this step in our hypothesis has the least supporting data as there is yet no direct evidence of the transfer of calcium ions from mineral to protein against the concentration gradient.

However, there is no evidence that this cannot occur. The second step in this hypothesis is the equilibration of calcium attached to the protein with the ionic calcium in solution. While this step has not been demonstrated to occur in bone fluid, it is a naturally occurring process throughout the body. The proposed process would permit the maintenance of a basic level of calcium in plasma and the ECF. The hypothesis is based on two important characteristics of calcium; the low solubility of its phosphate salts and its ability to complex reversibly with most proteins. These two characteristics permit the development of extracellular processes that are physiochemical in nature and require little energy.

We conclude this report with an appraisal of the teleological significance and necessity for the existence of such a proposed process. Calcium provides a cation that is vital for the structure and metabolism of organisms. Neuman and Neuman once proposed that calcium, because of its ability to associate with other molecules, may have been the precipitating nucleus for the origin of life[14]. There is a more obvious role for calcium in vertebrate evolution: to simultaneously provide a living endoskeleton and enable the maintenance of sufficient calcium in the circulation to sustain life. The calcium gradient from blood to bone produced by the internal skeleton necessitated a process for the return of calcium to blood. This return process is indispensable for life and deserves serious study. Once this mechanism is established, the precise role of parathyroid hormone should easily be determined.

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