Hypothesis

Extraosseous calcification in patients with chronic renal failure—no escape?

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Paradigms

Paradigms provide frameworks for thinking about and describing the nature of reality. They allow us to infer causal relationships between observations, make predictions about future developments and, in the field of medicine, devise strategies for achieving therapeutic aims.

Let me begin by following the advice of the geneticist Luigi Luca Cavalli-Sforza, who stated: ‘To understand the present, you have to understand history, and to understand biology you have to understand evolution, because evolution is the history of biology’. Exactly a century ago, MacCallum described the enlarged parathyroid glands of renal failure patients. Thirty years later, Albright observed the related changes in bone histology now known as osteitis fibrosa. Twenty years of therapeutic trials with vitamin D ended with the remarkable sentence of Stanbury, in a Lancet paper published in 1960: subtotal parathyroidectomy is necessary since ‘therapy with vitamin D cannot be relied on to cause involution of the enlarged and possibly autonomous parathyroid glands’. At the end of the 1960s, clinicians’ ability to measure parathyroid hormone (PTH) led to the therapeutic goal of lowering the levels of this hormone, as high levels of secretion by the parathyroid gland had been identified as the cause of secondary hyperparathyroidism. This advance initiated the therapeutic use of activated vitamin D analogues.

Compartment models

In the early 1990s, my colleagues and I began carrying out routine echocardiographic examinations of all haemodialysis patients being treated at the KfH-Nierenzentrum, Nürnberg, Germany. While increasing calcification had been observed in most of these patients after several years, echocardiography did not allow exact measurements of either the volume changes or the density of these mineral deposits. Luckily, at that time I met David Faul, a physicist at Siemens Medical Solutions (Erlangen, Germany), who was developing a new, very fast, X-ray system (Evolution; Siemens), with the goal of bringing it to market for use in coronary screening. However, the coronary calcification scores of the patients he had tested were very low, and he was interested in determining the accuracy of the system in patients likely to have higher scores. I therefore suggested that we use his X-ray system to examine haemodialysis patients at the KfH-Nierenzentrum.

As we began to find a correlation between calcium and phosphate (Ca/P) concentrations in serum and the product of the density \( C^2 \) volume of coronary calcifications, it became clear that a new, kinetic model was needed to describe this relationship, and that in order to describe the fate of minerals arriving at sites of extraosseous calcification, it would have to consist of five compartments: (i) gut, (ii) intravascular, (iii) kidney, (iv) bone and (v) extraosseous (Figure 1).

In addition, to describe the relationship between the total concentration of Ca/P ions and the amount of Ca/P at a single site in the extraosseous compartment (Equation 1), the binding of these minerals to protein would also have to be considered in order to explain what mass per time moves from one compartment to the other, as such fluxes involve only the free, but not the bound fraction of the minerals. Thus, determining the availability of the substrates would require subdividing the intravascular compartment into the protein-bound and free compartments. However, this would have made things extremely complicated, as the mathematics of an open five-compartment model consist of a collection of differential equations, and it would have been practically impossible to provide the many parameters necessary to solve these equations. Thus, while an open five-compartment model most closely approximates the real-life situation, it cannot be used when simultaneously considering the three...
fundamental processes involved in secondary hyperparathyroidism: (i) accumulation of Ca/P in the intravascular compartment, (ii) distribution of Ca/P between the bone and extraosseous compartments, and (iii) correction of Ca/P in the intravascular compartment by the kidney.

So far, most investigators have examined only the relationship between PTH and the Ca/P concentration in the intravascular compartment under defined interventions, including calcium supplementation, low or high phosphate diet, and medications such as vitamin D analogues. In the absence of a model of extraosseous calcification, it has therefore been difficult to distinguish the cause of this disease from its consequences. However, by eliminating the kidney compartment, thereby mimicking the situation of a patient with untreated end-stage renal disease, who is essentially anephric, a model of secondary hyperparathyroidism can be proposed. The result was that the model changed from an open five-compartment model to the closed four-compartment model shown in Figure 2, as in an anephric patient the Ca/P ions that reach the intravascular compartment are no longer able to exit the system, i.e., the body, via the kidney.

In the intravascular compartment, the masses of Ca/P are the concentrations of these minerals x the volume of the compartment. If the latter is constant, the concentrations of Ca/P are equivalent to their masses. The question then arose: when during renal failure do the concentrations—or, assuming a constant volume, the masses—of Ca/P increase in the intravascular volume? In Martinez et al.'s [1] study of patients with early renal failure, the increase in the phosphate and calcium concentrations started at a creatinine clearance of 59–50 ml/min and 29–20 ml/min, respectively. The time shift between the increases in the mass of phosphate and that of calcium can be explained as follows: in patients who still have functional kidneys, a balanced but dynamic state exists between the intravascular compartment and the four other compartments. Thus, increased phosphate in the intravascular compartment indicates an imbalance between absorption by the gut, precipitation and solubilization from the extraosseous compartment, influx and efflux from the bone compartment, and excretion by the kidney. To prohibit a steady increase of the phosphate mass in the intravascular compartment, phosphate must exit by at least one of three ways: (i) transfer to the bone compartment, (ii) extraosseous calcification, and (iii) excretion by the kidney (excretion by the gut is negligible).

The accumulation of phosphate stimulates the secretion of PTH, which occurs simultaneously with the increase in intravascular phosphate mass. PTH mobilizes Ca/P from the bone compartment and thus, according to the law of mass, drives the formation of calcium–phosphate molecules either into the intravascular or extraosseous compartment or in the bone compartment.

Calcium is a weakly acidic cation with an equilibrium constant (pKa) of 12.8. In the body, it often exists in the hydrated form, which allows it to be passed selectively across various physiological barriers.
The hydrated oxo-anions of phosphorus undergo hydrolysis, yielding basic solutions. Between pH 2.8 and 7.3, $[\text{H}_2\text{PO}_4^-]$ predominates, while from pH 7.3 to pH 11.8 $[\text{HPO}_4^{2-}]$ (aq) is formed. Higher pH values lead to the formation of $[\text{PO}_4^{3-}]$. When the products of the concentrations of certain cations and anions exceed the solubility products of their salts, insoluble precipitates are formed. Moreover, while the solubility of ionic salts in water presents certain complexities, the situation becomes even worse in the aqueous solutions of the tissues, where acidic cations, such as calcium, and basic anions, such as oxo-anions of phosphorus, combine to precipitate as insoluble salts. Although the combination of weakly acidic calcium and moderately basic phosphate strongly favors insolubility, there is, nonetheless, also some degree of solubility. In addition, the solubility of salts of basic anions is enhanced in the presence of strong acids, whereas bicarbonate overload results in precipitation.

What is the preferred way to lower the accumulated amount of phosphate in the intravascular compartment? This is not an easy question to answer, as again the parameters describing the dynamic behaviours of the masses as they move between the compartments are unknown. However, we can carry out the following thought experiment: in the four-compartment model shown in Figure 2, a single phosphate ion has been absorbed and has reached the intravascular compartment. That ion can proceed to either the bone compartment or the extraosseous compartment, but never to both compartments at the same time. If this is true for a single ion, then it should be valid for all phosphate ions at the same time. If the two compartments were homogeneous spaces with the same physiological characteristics and the same volume, the masses of the substrates would be divided equally among them, powered by osmotic forces, until a state of equilibrium were reached. But this is not so, instead there is strong inverse relationship in patients with secondary hyperparathyroidism between the masses accumulating in the two compartments: the higher the content of phosphate ions in the extraosseous compartment, the lower the content in the bone compartment. In keeping with this theory, an inverse relationship of the mineral density between the coronary–artery compartment and the bone compartment has been shown [2,3]. Finally, what is true for phosphate should also be true for calcium, which follows the pathways of phosphate at the same rate, as calcium–phosphate is formed.

Where within the extraosseous compartment does precipitation occur? This depends on the proximity of a particular molecule or group of tissue-related molecules, their hydrolytic activities, and the local physicochemical conditions which, in turn, are modified by a variety of local events, such as hormone action, inflammation, disturbance of binding, structural changes in response to blood pressure, apoptosis, the formation of cellular waste products and medications. The vascular wall is particularly exposed to calcification as it is the part of the extraosseous compartment closest to the intravascular compartment and therefore the one most quickly accessible by diffusion. There is, however, constant competition between other components of the extraosseous compartment and the vascular wall. In addition, precipitation itself generates osmotic forces on molecules directed to the site of precipitation. The sum of these forces becomes zero if all agonistic and antagonistic factors at a single site are equal to those at all other sites—a very unlikely situation in a biological system. Instead, osmotic forces

![Four-compartment model of Ca/P kinetics in an anephric patient. During bone growth, bone mineralization is the preferred route of Ca/P utilization, with only a small amount of extraosseous calcification. At the end of bone growth, there is increasing extraosseous calcification and, as a consequence, demineralization of bone under the influence of PTH. For details, see text.](image)

**Equation 2.** Law of mass.

$$10\text{Ca}^{2+} + 6\text{PO}_4^{3-} + 4\text{H}_2\text{O} \longrightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 2\text{H}_3\text{O}^+$$
in the opposite direction of the site of precipitation favour solubilization of the precipitate.

Keeping this series of events in mind enables us to understand the events leading to coronary calcification. Using the same electron-beam computed tomography technique that we used 4 years ago, which showed high levels of coronary calcification in haemodialysis patients, Goodman et al. [4] found that coronary calcification scores in end-stage renal disease patients younger than 20 years were very low but increased tremendously above that age. To understand the onset of calcium precipitation in the coronary arteries, it is necessary to examine the events that take place in the bone compartment. Bone is a very complex tissue whose remodelling is regulated by osteoblasts and osteoclasts. These two cell types are closely interrelated, as osteoclasts can only differentiate in collaboration with osteoblasts. Therefore, based on the functions of these cells, the bone compartment can be subdivided into: (i) the osteoid compartment, whose matrix is synthesized by osteoblasts, and (ii) the mineralized compartment, where osteoclasts release both HCl, which dissolves Ca/P, and the enzyme cathepsin, which breaks down the osteoid matrix. In growing bone, the affinity of osteoid for Ca/P is much higher than that of any tissue in the extraosseous compartment. However, when bone growth stops, there is no further need for a net transport of Ca/P into the bone compartment. Instead, the processes of bone remodelling become predominant, and they continue until the end of life. After the age of 30–40 years, these dynamic changes in bone are accompanied by a continuous loss of Ca/P, which may be aggravated by hormonal changes, malnutrition, inflammation and immobilization. Goodman et al. [4] made a second important observation: the coronary calcification scores in their study subjects were much higher than those of our patients; their patients had an intake of elemental calcium of about 6500 mg/day compared to about 2250 mg/day in our patients. Apparently, regarding extraosseous calcification, it makes no difference whether calcium or phosphate drives the law of mass in the direction of precipitation.

Extraosseous calcification in renal patients with adynamic bone disease is less rapidly progressive than in those with secondary hyperparathyroidism, as only the amounts of Ca/P absorbed by the gut generate osmotic pressure according to the concentration gradient in the direction of extraosseous precipitation. Furthermore, there is little or no dynamic activity caused by the oversuppression of PTH, such as occurs with excess vitamin D intake, excess aluminium accumulation on the surface of mineralized bone, or the inappropriate use of calcimimetics, with essentially no release of Ca/P from bone. In terms of ion exchanges between compartments, adynamic bone disease is equivalent to elimination of the bone compartment. In this case, exogenous Ca/P excess, that is the amount of absorbed Ca/P exceeding the uptake capacity of the intravascular volume, inevitably precipitates in the extraosseous compartment, as shown in Figure 3.

Conclusions

There is no calcification without calcium and phosphorus. They are ingested and absorbed from the gut, and the only route of elimination is the kidney. The amount of phosphate absorbed by the gut minus the amount excreted by the kidney first accumulates in

![Fig. 3. Three-compartment model of calcium–phosphate kinetics in a patient with chronic renal failure and adynamic bone disease. The five-compartment model has changed to a three-compartment model due to the loss of kidney function and the dynamic activity of remodelling of bone (adynamic bone disease), both of which lead to increased extraosseous calcification.](image)
the intravascular compartment. Powered by osmotic forces, phosphate diffuses out of the intravascular compartment and induces, according to the law of mass, extraosseous precipitation. As a result, osmotic pressure is exerted on calcium to move to the site of precipitation. The intravascular calcium level is corrected by increased secretion of PTH and changes in Ca/P mobilization from bone; calcium and phosphate cannot egress separately from bone.

In a patient with chronic renal failure, who is unable to excrete Ca/P normally, conclusions 2 and 3 establish a ‘perpetuum mobile’, a self-perpetuating mechanism. The greater the impairment of the only correcting organ, the kidney, the worse the situation becomes. In such patients, secondary hyperparathyroidism ensues as an adaptive process.

Ca/P precipitate in all tissues but to varying extents, depending on the balance of local factors that promote or inhibit precipitation. The affinity of osteoid for Ca/P is much higher than that of all other tissues.

The intravascular compartment is protected against precipitation of circulating compounds by circulating factors, especially proteins, which bind a wide range of substrates. Protein binding allows the transport of large masses with only a small risk of reaction. Disturbances of protein binding result in catastrophic intravascular precipitation such as calciphylaxis.

There is an inverse relationship between the accumulated masses of Ca/P in bone and in extraosseous compartments. If the normal route of renal elimination is no more available, only a continuing dynamic process of distribution between these two compartments is possible.

**Implications**

Every intervention that increases the masses of Ca/P in the intravascular compartment of anuric patients increases the risk of extraosseous calcification if osteoid cannot act as a buffer for these substrates. In this condition dietary guidance aimed to reduce oral phosphate intake is extremely important.

Vitamin D (25-OH vitamin D₃) therapy should be reduced to the administration of a physiological dose, as it promotes the absorption of Ca/P and osteoblast differentiation; it may therefore increase the buffering capacity of the bone compartment and mineralization. Active vitamin D analogues should be avoided or used only at physiological doses, which have to be carefully chosen in patients at risk of extraosseous calcification. Administration of pharmacological doses increases this risk by increasing the amounts of Ca/P that accumulate.

The use of calcium carbonate or calcium acetate as a phosphate binder should be reduced such that the mass of calcium in the intravascular compartment is not increased at any time.

Regular use of non-calcium-containing phosphate binders is necessary to avoid an increased mass of phosphate in the intravascular compartment. Alternatively, or in addition, the weekly frequency of dialysis treatment should be increased (for example, daily dialysis) to correct hyperphosphataemia.

These are methods to avoid Ca/P accumulation and extraosseous calcification. They are also the only ways to avoid secondary hyperparathyroidism and renal osteodystrophy, as both conditions are associated with extraosseous calcification.

What is the optimal dose of elementary calcium that avoids secondary hyperparathyroidism and bone mineral loss? An increase of phosphate in the intravascular compartment at the rate dP/dt leads to efflux into the extraosseous compartment at the same rate following osmotic forces along the concentration gradient. This, in turn, results in the precipitation of phosphate with extraosseous calcium which, according to the law of mass, generates a flux of calcium at the rate 5/3 dP/dt from the intravascular compartment into the extraosseous compartment. To restore intravascular calcium, which is maintained at a constant level, this amount must be mobilized from bone—a process that requires PTH secretion—if no additional calcium absorption takes place at the same time. However, PTH mobilizes phosphate at a rate implying that there is never an imbalance between calcium and phosphorus in the bone compartments.

In the presence of excess phosphate accumulation, the rate of extraosseous calcification is less if the dialysate calcium is higher than 1.25 mmol/l or if the patient receives oral calcium supplementation. Under these circumstances, less calcium must be mobilized from the bone, as shown in Equation 3, and therefore less additional phosphate is added to the intravascular compartment via mobilization from bone, which again would stimulate extraosseous calcification.

The rate of mobilized calcium from the bone (dCa₅/dt) is a function of PTH secretion. Therefore, the rate of PTH secretion in an anuric patient with intact calcium regulation is as shown in Equation 4.

How should patients who already have Ca/P accumulation, extraosseous calcifications, elevated PTH levels and renal osteodystrophy be treated? Extraosseous calcification in an anuric patient (Figure 2) is decreased by changing the distribution between the extraosseous and bone compartments, which means finding a way to restore Ca/P to the bone. Alternatively, lowering the masses of Ca/P within the intravascular compartment drives the law of mass in the direction of removing Ca/P from the extraosseous.

$$\frac{dCa_b}{dt} = \frac{5}{3} \frac{dP}{dt} - \frac{dCa_a}{dt}$$

Equation 3. Relationship between calcium mobilized from the bone (Ca₅), accumulated phosphate (absorbed from the gut minus dialytic elimination) and accumulated calcium (absorbed from the gut and dialytic load) in the intravascular compartment.

$$\frac{d(PTH)}{dt} = f\left(\frac{5}{3} \frac{dP}{dt} - \frac{dCa_a}{dt}\right)$$

Equation 4. Relationship between PTH secretion and intravascular phosphate and calcium.
compartment; for example, with a dialysate calcium of 1.00 mmol/l and daily haemodialysis to sufficiently lower the intravascular mass of phosphate. However, the latter option is only possible using a moderate progressive approach, since lowering the mass of calcium in the intravascular compartment immediately increases PTH, with the consequence that Ca/P are released from the bone compartment.

What is the possible role of calcimimetic drugs in this scenario? Can we treat patients over the long term with low dialysate calcium and more frequent dialysis, concomitantly with the administration of calcium-sensing receptor modulating agent, so that PTH secretion is not triggered and mineral mobilization from bone is avoided? The results of a 2 year study evaluating the effects of administering up to 180 mg of cinacalcet once a day showed that it is possible to decrease PTH levels in a constant fashion over a period of 108 weeks [5]. However, while there was an initial rapid decline of Ca/P in the intravascular compartment, 26 weeks after the start of the treatment, the calcium × phosphate product had again slightly increased towards a level still below baseline values. This might have been due to high affinity of newly available osteoid for Ca/P in the presence of lowered PTH levels. As a result, Ca/P would have been extracted from the intravascular compartment to an extent that absorption from the gut and solubilization of precipitates in the extraosseous compartment could not have compensated for the deficit induced in the intravascular compartment. Once the osteoid was mineralized, the calcium × phosphate product would have began to increase again. This explanation is in good accordance with changes of intravascular Ca/P observed in patients after parathyroidectomy.

If the paradigm described herein is true, after the start of cinacalcet therapy in a patient with Ca/P accumulation, extraosseous calcification, elevated PTH and renal osteodystrophy then there should be a decrease of Ca/P in the intravascular compartment and a decrease in extraosseous calcification as a consequence of intensified mineralization of osteoid. After about half a year, there would be no further reduction but again an increase in extraosseous calcification if the systemic accumulation of Ca/P is allowed to continue; however, the rate of accumulation would be notably lower than before the initiation of treatment, because the additional mobilization of Ca/P from bone is now blocked by the action of the calcimimetic agent. This series of events has implications for future treatment schedules of chronic renal failure patients, the planning of related clinical studies, and the interpretation of studies of bone mineralization and the therapy of extraosseous calcification.

Generalizations

In the adult, after the end of bone growth, if intestinal phosphate absorption exceeds renal elimination—due to high phosphate intake or limited phosphate excretion—its subsequent accumulation in the intravascular compartment may trigger extraosseous calcification. Furthermore, if the elimination rate exceeds the absorption rate, as occurs with low phosphate intake, there is resolution of the extraosseous calcification, according to the law of mass.

Ca/P are absorbed simultaneously under the influence of vitamin D and other factors, their simultaneous mobilization from bone occurs in response to PTH release, whereas the kidney corrects Ca/P levels by increasing phosphate elimination and calcium reabsorption, also under the influence of PTH. Ca/P kinetics are strongly directed towards the formation of bone during the period of bone growth and towards maintaining the calcium ion concentration at a constant level, to optimize muscular and cellular functions so that they are preserved under sparse nutritional conditions with low Ca/P intake—a strategy that has clear evolutionary advantages.

After the period of bone growth has ended, in the young adult, an excessive intake of phosphate, one that exceeds the capacity of the kidney to adjust phosphate levels in the intravascular compartment, results in extraosseous calcification, which probably has implications for ageing and mortality. The mechanism of extraosseous calcification and hence coronary artery calcification is the same in renal and nonrenal patients, but coronary calcification scores are four to five times higher in chronic renal failure patients [2]. Thus, accumulation of Ca/P may well be the starting point of extraosseous calcification, and secondary hyperparathyroidism and osteodystrophy are consequences rather than underlying causes.

Conflict of interest statement. J Braun has served as a speaker for Amgen and Genzyme and is on the advisory board of Amgen.

References


