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# Physiopathology of osteoporosis and action mechanism of PTH

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## Osteoporosis. Concept

There is no universally accepted definition of osteoporosis. One of the most convincing is that proposed by the NIH in 1993<sup>1</sup>, according to which osteoporosis is a generalised disease of the skeleton, characterised by a reduction in bone mass and a deterioration in the microarchitecture of the bone, which results in an increase in bone fragility and a greater tendency to fractures. The loss of bone mass and the deterioration of the microarchitecture are consequences of an alteration in the phenomenon of bone renewal, whose fundamental protagonist is what is called a "unit of bone remodelling". Osteoporosis is therefore, ultimately nothing but a functional alteration in this unit.

Subsequent to the NIH definition, it has been felt necessary to introduce a new concept, that of bone quality<sup>2</sup>, which was not reflected in it. This concept includes both those aspects related to bone structure as well as those related to the characteristics of bone tissue (intrinsic properties of bone material). It is possible that an alteration in the quality of bone material is involved in the tendency to osteoporotic fractures, but in general its importance—in relation to bone mass and to those structural aspects—is less. Thus it can be accepted that the definition indicated earlier continues to be valid.

## Bone remodelling

The skeleton is an organ of support, and as such it is exposed to the processes of deterioration which all structures which have to bear mechanical load suffer. But differently from inert support structures (columns, beams, etc), bone is a living organ, with capacity for renewal, and for maintain-

ing its conditions of resistance. This renewal takes place in a permanently and has been given the name "bone remodelling"<sup>3,4</sup>. The speed at which it occurs is known as "bone turnover".

The aforementioned unit of bone remodelling, and that responsible for this phenomenon, consists in a group of cells charged with destroying small portions of bone, which are subsequently substituted by new bone. These cells are of various types, but two of them are the principal protagonists of the process: the osteoclasts (charged with destroying the bone), and the osteoblasts (charged with forming it). They intervene by lending their support to other cells such as lymphocytes, macrophages, endothelial cells, neurons, etc. The volume of renewed bone for each unit is 0.025 mm<sup>3</sup>, and the annual rate of renewal of the skeleton is approximately 10% (25-30% trabecular bone and 3-4% cortical).

In the skeleton, at any one time, there are more than a million active units. These units are out of step: some are found in the initial phase, others in the final phase, and others in different intermediary phases. There is a temporal asymmetry between the intervention of the osteoclasts and the osteoblasts. The former carry out their destructive task in some 2-3 weeks, while the latter take 4-5 months to replace the destroyed bone. Between the action of both types of cells there is an intermediate "investment" phase which lasts some 2 weeks. In this phase, cells whose origin is not well determined—although probably from the osteoblastic line (not macrophagic, as had been thought previously)—clean the bone surface produced by resorption, preparing it for the formative phase.

The spatial organisation of the units of remodelling varied according to whether they are in the cortical or trabecular bone<sup>7</sup>. In the first case the osteoclasts act on the sinus of the bone, moving longitudinally as they carry out their resorptive activity. Therefore the result of their action is a tunnel-shaped cavity. After the osteoclasts, the osteoblasts advance, closing this cavity. They do this by forming cylindrical and concentric bone layers, disposed from the walls of the cavity to its centre. The result is what are called “osteones” or bone structural units, which in the case of cortical bone is also known with the name of “Havers’ system”.

In trabecular bone the osteoclasts act on the bone surface, and do not move in a longitudinal way, but in an erratic zig-zag, in which the cell returns to pass over the area on which it has already acted earlier. The final result of its action is a cavity with a morphology which resembles a lagoon. The osteoblasts also fill this with layers from the bottom to the surface. The cavity, once filled, has in section, an aspect of a half-moon. This half-moon constitutes the bone structural unit or osteone of trabecular bone (some authors refer to these as “hemiosteones”, to compare the half-moon shape with the cylindrical shape of the layers of the Havers’ system). The endostic surface can also show units of remodelling with these characteristics.

The process of remodelling, together with its primary function of permitting the skeleton to maintain its characteristics as a support organ, is at the service of other biological phenomena of great interest. On the one hand, it allows the modification of the shape of the bone, to adapt it to changes in mechanical demands. In addition, it plays a role in the regulation of calcemia. On the other hand, it has shown its importance in the maintenance of the haematopoietic stem cells, located in the bone medulla next to the trabecular surface. Finally, its intervention in the homeostasis of acid-base equilibrium, as been indicated.

Before leaving this section on of bone remodelling, we should indicate the existence of another process, known as “modelling”<sup>6</sup>, essentially functioning during the development, and determining the morphological and structural transformation of bone throughout it. It consists of bone formation not preceded by resorption in certain places (fundamentally the periosteum, with which the external diameter of the bone increases) and with resorption in others (the endosteum, to increase the medullar cavity, and some zones of the periosteum –those that ought to transform metaphysis to diaphysis–). In adult life the subperiostic formation and the endostic resorption are maintained but with much lower intensity. This supposes a displacement of bone towards the outside (away from the central axis), which increases bone resistance and partly neutralises the deterioration which the bone suffers with aging. Some authors consider the concepts of “modelling” and “bone formation not preceded by resorption” as practically synony-

mous, and although this assertion is not always correct, it is most of the time.

In a healthy adult 97% of bone formation is due to remodelling, and only 3% to modelling.

### Bone remodelling unit cells

We have already mentioned that the principal cellular protagonists of the units of bone remodelling are the osteoclasts and the osteoblasts.

#### 1. Osteoclasts

The osteoclast is a multinuclear cell, the product of the fusion of mononuclear precursors (preosteoclasts), through the participation, amongst other factors of the protein DC-STAMP. Its origin is haematopoietic, deriving from a cell which is the common precursor of the osteoclast and the macrophage. To destroy bone it adopts a special shape<sup>7,8</sup>, by virtue of which the part of the membrane of the cell which comes into contact with something adopts a rugous character, which in histological images is described as a brush border. This border is made up of microvilli which discharge hydrogen ions and enzymes (principally cathepsin K) capable of destroying bone. The hydrogen ions eliminate the mineral component, and the enzymes the collagen. In order that these substances remain between the osteoclast and the surface of the bone developing their function, at the edge of the rugous zone they form a ring whose surface has the property of bonding closely with the bone; the result is that in the interior of this ring a sealed space is left, from which the hydrogen ions and the enzymes cannot escape. The ring is mainly made up of actin, and the reason why its surface appertains to the bone is that it possesses molecules of integrin  $\alpha v \beta 3$ , which tend to bond to RGD (arginine-glycine-aspartic acid) present in various bone proteins (vitronectin, fibronectin, osteopontin). In both the formation of the actin ring and that of the rugous surface, the cell cytoskeleton plays a key role. The configuration which this adopts, in addition, points the way to some cytoplasmic vesicles which direct the microvilli to release their contents into the sealed space, where they will exert their bone-destructive effect.

In the development of the osteoclast and in its functional activation a surface receptor called RANK is key. On the action of a molecule known as the “RANK ligand” or RANKL<sup>9</sup>, present in the membrane of osteoblastic line cells (precursors of the osteoblasts, and mesenchymal cells of the medullar stroma). The RANK-RANKL interaction involves therefore direct cell-cell contact. However, RANKL is occasionally present in soluble form. For the activation of the osteoclast it is necessary to combine RANKL with another molecule, M-CSF, for which the osteoclast also has a specific receptor (c-fms). M-CSF is also formed by osteoblastic line cells, but is not bonded to the cell membrane, but is a soluble factor. The osteoblastic line cells, in addition to these substances, produce others for which there are also receptors in the osteoclasts (e.g. OSCAR, TREM2)<sup>10</sup>, which are

considered “co-stimulators” with respect to the RANK-RANKL system. On the other hand, also acting on the osteoclasts are substances originating in other types of cells; of these one type are activators (TNF, VEGF-C) and others inhibitors (calcitonin). The interaction of the osteoclast itself with the bone matrix increases its survival.

The stimulation of the osteoclasts by RANKL gives way to the activation of various intracellular signalling pathways (NF $\kappa$ B and various MAPK)<sup>11,12</sup>, with the production of different factors, of which one, NFATc1<sup>13</sup> should be especially mentioned due to its importance. The action of the co-stimulators (ligands of OSCAR and TREM2) activates pathways in which there are present adaptor molecules such as ITAM, phospholipase C (PLC), calmodulin and calcineurin. Curiously RANKL can negatively regulate the formulation of osteoclasts.

A protein characteristic of the osteoclasts is tartrate-resistant acid phosphatase (TRAP), whose physiological role is not well defined.

The osteoclasts are involved in other functions, as well as, strictly, in osteoresorption. Some have to do with bone homeostasis itself, in their capacity to stimulate osteoblasts, to which we will return later. On the other hand they regulate the egress from the bone medulla of the haematopoietic stem cells (in which are involved certain receptors and the secretion of proteolytic enzymes), and may be involved in immune phenomena in inflammatory processes.

## 2. Osteoblasts

The osteoblasts have a mesenchymatous origin, and possess common precursors with cells such as fibroblasts, myocytes or adipocytes. The differentiation to osteoblasts from their precursors requires the presence in them of transcription factors runx 2, osterix, ATF4 (or CREB 2) and AP1 (heterodimeric transcription factor composed of proteins of the families Fos and Jun). They possess a powerful ribosomal apparatus, which is consistent with the intensive synthesis of protein which they perform. Of these, the most important, quantitatively, is collagen, but they also synthesise other proteins whose function is not always well known, among which should be mentioned, since it is the most known, is osteocalcin. As well as synthesising proteins the osteoblast drives bone mineralisation. The non-mineralised bone tissue, called “osteoid”, is formed by layers which are synthesised from the bottom to the surface, defined by the different orientation of the collagen fibres in each of them. Their mineralisation is carried out progressively from the deepest layers to those on the surface, after a period of osteoid maturation. Alkaline phosphatase is an osteoblast protein which is involved in the process of mineralisation, destroying one of its inhibitors, pyrophosphate, by which it also increases the local concentration of phosphate.

Rather surprisingly, the osteoblast has, along with the bone forming function which we have just commented on, a regulatory function on bone

destruction. According to what we have already said, the osteoblast –or its precursors– have the capacity to produce substances which stimulate the osteoclast. RANKL is the most characteristic, although not the only one. The osteoclast, in addition, produces a substance –osteoprotegerin (OPG)<sup>9</sup>– which has an affinity with RANKL itself, such that it binds with it, preventing it from accessing RANK, and, therefore, preventing the stimulation of the osteoclast. Ultimately, the behaviour of the osteoclast varies with the RANKL/OPG relationship. Many factors which act on the osteoclast (PTH, 1.25 (OH) 2D, oestrogens...) do so, at least in part, indirectly, through the osteoblast, modifying this RANKL/OPG relationship. The osteoblast does not only have the capacity to stimulate the osteoclast (a function which develops when the activity of a bone remodelling unit is initiated), but also to inhibit it (when the osteoclasts have to end their activity 2-3 weeks later), which they bring into effect through OPG, and the ephrin system<sup>14</sup>, to which we refer in more detail later (the osteoblast has something in its membrane called EphB4, which, by bonding with the Ephrin B2 present in the osteoclast’s membrane, slows it).

Now we intend to focus on the bone-forming aspects of the osteoblast. The principal signalling pathway involved in this –although not the only one– is considered to be the Wnt- $\beta$ catenin system<sup>15,16</sup>. The proteins Wnt have available a receptor on the surface of the osteoblasts, called Frizzled, for which there is a co-receptor (LRP5). When these proteins bond to the Frizzled-LRP5 complex, they are prevented from acting in a conjunction with cytoplasmatic proteins whose function is to phosphorylate the  $\beta$ catenin, so that is degraded in the proteosome. By avoiding this phosphorylating effect the  $\beta$ catenin accumulates in the cytoplasm and passes into the nucleus. Here, an increase in the transcription factors “T cell factor/lymphocyte stimulator factor” (TCF/LEF) takes place, which stimulates the genes involved in bone formation, including runx 2. Other substances which stimulate bone formation by the osteoblast are the bone morphogenetic proteins (BMP), TGF $\beta$ , IGFs, FGF, PDGF, endothelin, PTHrP, etc.

The Wnt- $\beta$ catenin pathway establishes a nexus between the bone-forming and anti-osteoclastogenic functions of the osteoblasts, since  $\beta$ catenin is involved in the regulation of the RANKL/OPG equilibrium, biasing it in favour of the latter. In general terms it can be said that the activation of the pathway in early phases of the life of the line cells induces formation, whilst in the later phases it reduces osteoclastogenesis. It has been suggested that the ligand of LRP5 decides which of the two functions should predominate.

Along with the stimulatory signals for bone formation the inhibitors should also be mentioned, the first ones of note, being those which antagonise the Wnt- $\beta$ catenin pathway, such as SFRP-1 (Secreted Frizzled-Related Protein 1), Dickkopf 1 (DKK1) or sclerostin, to which we will return later. It should also be mentioned that they are

inhibitors of intestinal serotonin, a function which has recently been described, and whose synthesis is regulated by ligands which act on the LRP5 of the enterochromaffin cells.

The osteoblast, after forming osteoid may remain carpeted to the surface of the recently synthesised bone (surface or coating osteoblasts), may stay buried in the sinus of synthesised bone in its surroundings (transforming itself into a cell called an "osteocyte", or dying by apoptosis. This last option is the one for which the majority of the osteoblasts are destined.

The osteocytes<sup>17,18</sup> have extensions which bind them to each other and with the surface osteoblasts by means of "gap junctions". It is considered that they perform a key role in bone remodelling, being involved both in setting in motion the bone remodelling units and in their termination. The former comes about by mechanisms which are poorly understood, but which are thought to consist in the detection of the changes produced in the bone (microfractures), due to which a signal is sent to the bone surface in order to activate the osteoclasts. The same thing happens in apoptosis. It is quite likely that the osteocytes are continuously sending to the bone surface inhibition signals for the osteoclasts (TGF $\beta$  and NO might carry out this function), and that what really happens is that after detecting a bone lesion they stop sending the signals. On the other hand, as we have said, the osteocyte seems also to be involved in ending the activation of the unit of bone remodelling once it has formed the necessary quantity of bone. This function would take place through the synthesis of sclerostin, a substance which would reach the bone formers on the surface where it would inhibit the Wnt- $\beta$ catenin system by binding with the LRP5 co-receptor or blocking it. However, there are still aspects to be clarified. For example, a rat model deficient in osteocytes has reduced bone formation, despite a lack of sclerostin cell formers.

Apoptosis of the osteocytes, as well as determining the initiation of bone resorption, itself causes an increase in fragility for poorly understood reasons. Among those phenomena determining apoptosis of the osteocytes which should be remarked upon, in addition to a lack of mechanical stimulus, is a lack of oestrogen and the glucocorticoids.

### Regulation of bone remodelling

Bone remodelling is subject to regulation by a series of factors which stimulate or inhibit the osteoblasts or osteoclasts, some of which have been mentioned in the description we have just given of these cells. We are going to consider them then, systematised in three sections: 1) factors involved in what is called the "osteoclast-osteoblast dialogue" (that is to say, how both types of cells relate to one another)<sup>19-22</sup>; 2) other local regulatory factors (products of cells of the bone micro-environment other than osteoclasts and osteoblasts); 3) systemic factors.

### 1) Factors involved in the osteoclast-osteoblast dialogue (Figure 1)

The specific factors which connect both types of cells, and how they connect, are largely unknown and, therefore, their description is, to a certain degree, speculative.

The first idea which should be taken into account is that the relationship between the osteoclasts and the osteoblasts is not static or constant, but that it changes across the different evolutionary phases of the unit of remodelling.

#### a) Initiation of the unit of remodelling. Bone destruction

As we have already indicated, is considered to happen when the osteocytes detect the necessity that a part of the bone needs to be renewed<sup>23</sup>, they send stimulatory signals (or stop sending inhibitory signals) to the bone surface, so as to initiate osteoclastogenesis. It is thought that these signals are received by osteoblastic line cells, which respond by synthesising chemotactic factors for osteoclast precursors (e.g. sphingosine-1-phosphate, osteopontin), producing RANKL and other substances which activate osteoclastogenesis and mature osteoclasts, and release collagenase which prepares the bone surface so that resorption can commence. The type of osteoblasts involved in these phenomena are not well known, but could be treated as coating osteoblasts or as mesenchymal cells; in either case it appears that they belong to a particular subtype of osteoblastic line cells which express ICAM-1. It is possible that RANKL and M-CSF could also be produced by their own osteocytes, and the possibility has also been considered that apoptic bodies of the osteocytes may increase the formation of the osteoclasts

It has been suggested also that the preosteoclasts attracted by the chemotactic agents to those sites where a unit of bone remodelling is going to be initiated, can be found lodged, and partially activated, in niches near to them, from which they would move towards them.

#### b) Investment phase. Coupling phenomenon

Once a sufficient quantity of bone has been destroyed, the activity of the osteoclasts (which then finally die through apoptosis) needs to be slowed, and the osteoblasts stimulated. The fact that the osteoblasts, and following them, are activated in the same place in which the osteoclast had previously acted, is a phenomenon known as "coupling", or a temporo-spatial fit between the action of the osteoclasts and that of the osteoblasts. The mechanisms responsible have not been established with certainty, but various possibilities have been considered, all compatible with each other:

##### I.- Substances released from the bone matrix

During the formation of the bone matrix, remaining buried in an inactive form, are substances synthesised by the osteoblasts themselves or from the circulation, which with bone resorption are released and activated, performing a modulating effect on the activity of the bone cells. The best known is TGF $\beta$ , which on the one hand inhi-

bits the osteoclasts, and on the other attracts (by a chemotactic effect) osteoblast precursors, and stimulates their proliferation. Other substances released from the bone matrix which are osteoblast stimulators are the IGFs, the BMPs, FGF and PDGF. There is disagreement about up to what point the proteolytic enzymes present in the sealed space contribute to their activation (acting on the inactive form) or to their inactivation (acting subsequently on their active form), such as is necessary to reach optimum levels.

II.- Release by the osteoclasts of substances which stimulate the osteoblasts

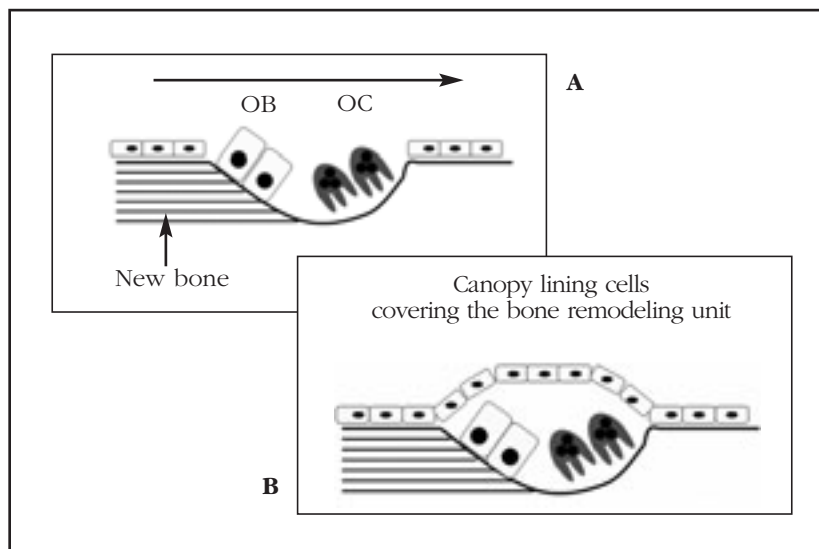
Although little known, included as a possible candidate in this section is cardiotrophin (which is indicated by glycoprotein 130).

III.- Mechanisms dependent on cell-to-cell contact

In the proximity of the osteoclasts there are osteoblastic line cells with which they establish contact. This contact appears to be supported by the existence of a layer of cells of this line (from the "coating" type of osteoblast) covering the space which the remodelling unit occupies, to which we usually refer as the "canopy". The cells of this canopy can be found near the osteoclasts. It is also possible that some canopy cells are precursors of the osteoclasts (a variety of macrophages which some authors call "osteomac" –from osteal macrophages–).

On the other hand, various blood vessels have access to the remodelling space bounded by the bone and the canopy, through which osteoblast precursor cells can access the central focus of resorption. It is possible, also, that they can do this directly from the bone medulla by means of these canopy cells, attracted by the factors which are released in the centre of resorption. Another factor which favours cell-to-cell contact comes from the fact that both the osteoclasts and the osteoblasts feature cytoplasmic extensions (similar to those of the osteocytes) which allows them contact even though their cell bodies are at a certain distance. Earlier we have indicated how, in the resorptive phase, the osteoblast-osteoclast connection results in the stimulation of the latter by RANKL produced in the former. In this second phase of evolution of the bone remodelling unit the RANKL/OPG relationship changes, and a displacement is produced in favour of the latter, in such a way that OPG is predominant and the osteoclasts are inhibited. This change is induced, at least partly, by the osteoclasts themselves. In fact,

Figure 1. Unit of bone remodelling indicating: A.- the successive intervention of osteoclasts (black) and osteoblasts (grey); B.- the canopy which covers the space being remodelled



the osteoblasts have a receptor in the membrane (Notch) for which the osteoclasts have various ligands, also situated on their membrane (Jagged and Delta). It is thought that the activation of the Notch receptor promotes the synthesis of Wnt proteins, which is probably stimulated also for other reasons not yet clear. The Wnt- $\beta$ catenin system determines the change in the RANKL/OPG relationship in the way we have already mentioned –in addition to stimulating osteoblastic differentiation–.

A relationship is established between the osteoclasts and the osteoblasts, as well as through another ligand-receptor system in which the elements are found in the membranes of these cells. This is the system of ephrins<sup>14</sup>. What is interesting about this system is that, when its two elements bond, signals are not only sent towards the cell which contains the receptor, but also towards that which contains the ligand. In the case that we are now discussing, the osteoblast is stimulated and the osteoclast inhibited. The osteoclast presents ephrin B2 and the osteoblast its receptor EphB4.

*c) Bone formation and ending of the action of the bone remodelling units*

Once the osteoblasts are activated bone synthesis occurs. This process appears to be self-fed, since the osteoblasts synthesise substances which stimulate themselves in the form of auto-cines (IGF, TGF, FGF, BMP...). One of the substances to which most importance is given today is PTHrP<sup>24,25</sup>, for which the osteoblast has a receptor (PTHrP1) which is common to this substance and to PTH. The bone stimulatory effect of PTHrP should be intermittent, for which its action needs to happen in a context of mechanism which is involved in determining this intermittence. Once the bone synthesis has produced an adequate quantity of bone, it must cease. This task appears

also to fall to the osteocytes. They receive some information (perhaps mechanical) by virtue of which they synthesise sclerostin which inhibits the action of Wnt proteins through their effect on the co-receptor LRP5. It is possible that other mechanisms are involved. Some of which may be of a physical nature: a mechanostat which detects when sufficient bone has been formed, or a topographic mechanism, to which we refer below, capable of detecting the fact that there are no longer any empty spaces on the bone surface.

#### IV.-Topographic mechanism

There are data which support the idea that the existence of a vacant space on the bone itself sets in motion the mechanisms for bone formation in relation to a phenomenon which detects the surface configuration or the spatial limits of bone tissues. Maybe aspects related to the distribution of mechanical load are involved.

### 2) Other local factors

At the margin of the factors involved in the osteoclast-osteoblast dialogue (local by definition), other factors synthesised in other types of cells also present in the bone microenvironment are involved in the regulation of bone remodelling: lymphocytes, macrophages, endothelial cells, and even the mesenchymal cells themselves (from which the osteoblasts derive). These factors are frequently co-determining. On the other hand, they can be capable of acting both on the osteoclasts as well as on the osteoblasts, in general in an opposing way (if they inhibit one, the other is stimulated), and therefore giving the same final result (either increasing or decreasing bone mass). Sometimes their action on the osteoblasts has repercussions on the osteoclasts by means of the RANKL/OPG system.

These factors are usually cytokines or growth factors<sup>26-28</sup>. Some cause a reduction in bone mass, as is the case with those called inflammatory cytokines – IL-1, TNF, IL-6 – which promote bone destruction, and others its increase, such as IL-4, the IGFs, BMP, TGF $\beta$ , PTHrP, etc.

### 3) Other systemic factors

General factors which intervene in the regulation of bone remodelling are usually classified as humoral (hormones) and mechanical.

#### I.- Hormones

- PTH.- Endogenous PTH appears, essentially, to have a stimulatory effect on bone destruction. This is, at least, the effect which has been confirmed for PTH when it is administered continuously. Such an effect is performed through the osteoblasts and their production of RANKL. On the other hand its intermittent administration stimulates bone formation<sup>29</sup>. The reasons for this difference are not well understood. We will return to the anabolic effect of PTH later.

- Oestrogen.- The oestrogens have a positive effect on the bone through multiple mechanisms<sup>30,31</sup>. On the one hand, there are receptors for them in both osteoclasts and osteoblasts, in the

second of which they bias the RANKL/OPG relationship in favour of the latter. On the other hand, they inhibit the production of osteoresorptive cytokines by the macrophages and the lymphocytes.

- Glucocorticoids.- The glucocorticoids, at physiological concentrations, have a permissive effect on bone formation. At pharmacological concentrations, however, they depress the activity of the osteoblasts and, initially, increase that of the osteoclasts, resulting in a reduction in bone mass<sup>32</sup>. The glucocorticoids reduce osteoprotegerin.

- Calcitonin.- Calcitonin is a powerful antiresorptive agent, although it may play some role in bone formation, since in knockout rats calcitonin results in an increase in bone formation<sup>33</sup>.

- Serotonin.- We have already indicated that serotonin has been revealed as a powerful inhibitor of osteoblasts<sup>34</sup>. Its synthesis takes place in the enterochromaffin cell, from where it is released into the blood, of which 95% passes into the platelets. The remaining 5% has access to the osteoblasts, which have receptors for it. Our knowledge of the effects of serotonin on bone are still at a very early stage.

#### II.- Mechanical factors

Mechanical load exerts a positive effect on bone, and its absence (weightlessness, being bedridden), a negative effect, increasing bone turnover and encouraging bone destruction. The mechanisms through which these effects happen are not fully known, but appear to involve the osteocytes<sup>35-36</sup>. The osteocytes would detect changes in the mechanical load through changes in the flow of liquid which surrounds the extensions in the canaliculi where they are sited, and through the stimulation of structures which bond the surface of the extensions with the walls of these canaliculi, in which integrins are presumably involved. Other studies suggest the involvement of ionic channels present in the membrane of the osteocytes. In whichever case, the stimulus detected by the membrane structures should transcend the cytoskeleton and activate intracellular signalling pathways (MAPK).

An increase in runx 2 and osterix, as well as  $\beta$ catenin, has been found in the osteoblasts of bone submitted to mechanical overload. This probably relates to the fact that the mechanical stimulus reduces the production by the osteocytes of sclerostin, antagonist of LRP5. The mechanical stimulus appears also to inhibit another antagonist of the Wnt pathway, Dkk1. In addition to the sclerostin-Wnt- $\beta$ catenin system, the response of the bone to mechanical stimulus appears to involve other substances, such as NO and the PGs. Also involved is the RANKL/OPG relationship, perhaps in relation to the modification in  $\beta$ catenin. Finally, an increase in osteopontin has also been detected, in whose absence (KO rats) bone remodelling produced in response to mechanical changes is diminished, which has been related to a possible chemotactic effect of protein for the osteoclasts.

PTH sensitizes the bone to the mechanical sig-

nals, which is what the fact that the anabolic effect of the mechanical stimulus is lost in rats subject to parathyroidectomy, appears to indicate. PTH inhibits sclerostin, exerting on it a synergistic effect with  $\beta$ catenin in response to mechanical stimulus. It should be taken into account that mechanical overload, although initially anabolic, when excessive can drive an increase in bone turnover with bone loss. This is because it can result in an accumulation of microcracks. Bone modelling (subperiosteal formation) however, does not seem to be negatively affected in this situation.

The response to mechanical stimulus reduces progressively if it persists in a steady manner, thus mechanical overload is more efficacious from an osteogenic the point of view of if it occurs intermittently.

### Alterations in the units of bone remodelling in osteoporosis

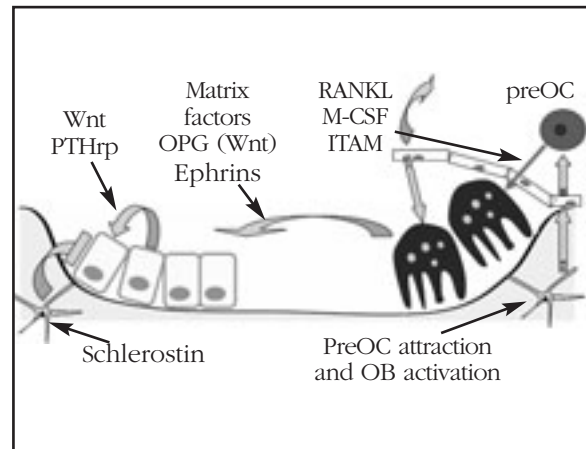
We have indicated at the beginning of this chapter that osteoporosis is a dysfunction of the unit of bone remodelling<sup>37</sup>. This dysfunction is due, essentially, to two types of alteration. The first consists of the establishment of what we know as “negative balance”; the second in an increase in the number of units of bone remodelling, which gives way to what we call “increase in bone turnover”. (Figure 2).

#### a) Negative balance

In the young adult the quantity of bone which makes up the osteoblasts in each unit of bone remodelling is equal to that which the osteoclasts have previously destroyed. The situation is known as “zero balance”. However, at around 40 years of age, or perhaps a little earlier, the quantity of bone formed by the osteoblasts starts to be a little less than that destroyed by the osteoclasts. This situation is described as “negative balance”. Given that, as we have already said, the number of units normally functioning in the skeleton is higher than a million, this means that from this age there are more than a million points in which bone mass is being lost. The result, logically, is the reduction in its total quantity. Depending on the initial bone mass, on the degree of negative balance, and the time during which it has been present (and certainly, the age of the person), this loss can take place at values of bone mass which qualify as osteoporotic. Negative balance is a sine qua non condition for the development osteoporosis.

Negative balance which develops with age is due fundamentally to a reduction in bone formation, probably related both to a decrease in the number of osteoblasts (due in part to a reduction in its precursors, in part to a diminution in their differentiation, and in part to a reduction in their survival) and in their individual activity. This, at least partly, is due also to falls in the bone micro-environment of the concentration of stimulator factors of these cells, which in one case (Wnt proteins) has been attributed to an increase in ROS radicals during aging. On occasion, an increase in bone resorption contributes to the negative balan-

Figure 2. Mechanisms involved in the bidirectional dialogue existing between osteoblasts and osteoclasts. See the text for a detailed explanation



ce, due to an increase in osteoclastic activity. This increase could translate into a greater movement of osteoclasts, up to the point that the trabecular could become perforated. On the other hand, this increase in the activity of the osteoclasts is accompanied by the birth of a higher number of bone remodelling units, so that a phenomenon known as “increased turnover” takes place, which is commented on in the following section. As opposed to the reduction in activity of the osteoblasts due to age, the increase in osteoclasts is related to the reduction in oestrogens. The lack of these hormones probably also inhibits the formative activity by favouring osteoblast apoptosis, which intensifies the negative balance.

#### b) Increase in bone turnover

The increase in the number of bone remodelling units when these are found in negative balance supposes an increase in the number of points in the skeleton in which bone mass is lost, and therefore, an acceleration of this loss. In fact, although the negative balance could be an essential factor in the development of loss of bone mass, the factor which is usually responsible for the greatest quantity of loss of bone mass is an increase in turnover. The forms of osteoporosis in which this factor effectively plays the main role is known as “high turnover osteoporosis”. The most characteristic example of increased turnover is that which constitutes the menopause, with the depletion of oestrogens which is brings. To this is due the acceleration of the loss of bone mass which follows it, and is ultimately the mechanism responsible for what is called “postmenopausal osteoporosis”. There can also be an increase in bone turnover in later ages of life which is usually attributed to an increase in PTH in relation to a reduction in renal function and the endowment of vitamin D. There are some forms of osteoporosis –less frequent– in which turnover is not increased, such as, for example, idiopathic osteoporosis in males.

### Consequences of alterations in bone remodelling units (Figure 3)

The differences in the structure and spatial positioning of the osteones in trabecular and cortical bone mean that the impact of the changes in the unit of remodelling on which we have just commented are different in the two sections of bone.

#### *a) Trabecular bone*

As a consequence of the negative balance, a reduction in bone mass is established which is translated primarily into a thinning of the trabeculae. On the other hand, the increase in turnover intensifies this thinning, that, along with a greater movement of the osteoclasts due to this situation, tends to cause trabecular perforation. The accumulation of perforations makes a large part of the trabeculae start to disappear, such that the morphological aspect of the trabecular framework changes from what is called a "plate pattern" to a "rod pattern". That is to say, from walls with holes in them, like a sponge or a honeycomb, to a kind of tridimensional lattice, with less capacity to support mechanical load. On the other hand, the same loss of trabecular material results in a disconnection between trabeculae, which reduces their support for one another, which diminishes even more their ability to support load<sup>38,39</sup>. Specifically, most of the trabeculae which are lost are those which are horizontal, for which reason the vertical ones which have been preserved lose their buttressing effect which they shared with the horizontal trabeculae by bonding with them. This means that the residual vertical trabeculae are, in functional effect, longer, which facilitates their curvature ("buckling") and, in the long run, their fracture.

Added to the fact that the trabecular framework consists of trabeculae which are thinner and poorly interconnected, is another phenomenon of interest: that of the "concentration of tensions" at the level of active units of bone remodelling. From when a unit of remodelling initiates its activity until it ends, a vacant space in the bone (corresponding to the bone which has already been destroyed but has not yet been substituted by the new formation) is generated. Its presence in the thin trabecula produces weak points in which all the tensions in this trabecula has to support are concentrated (called "stress risers")<sup>38</sup>. At this point a fracture of the trabecula is easily established in the same way that if subject to a load, a structure (for example, a stick) which has been thinned at a certain point, will tend to snap at that point. In situations of high turnover, given that the number of active units of remodelling in them is greater, so also will be the number of "concentrators of tension", and thus, the points in which there is a risk of developing a fracture. The free spaces in the bone due to their being renewed, determinants of the concentrations of tension, frequently known jointly as "spaces in remodelling", in the literature written in English, tend to be described as "transient remodelling (spaces)"<sup>40</sup>, to give an understanding that the loss of bone is reversible (transitory),

given that it disappears once the osteoblasts fill the hole formed earlier by the osteoclasts.

In men, the reduction in bone mass with age is not established at the expense of an increase in turnover (possibly in relation to the absence of a phenomenon equivalent to the menopause), rather of the negative balance, in such a way that their trabeculae, rather than suffering a process of perforation and disconnection, undergo thinning.

#### *b) Cortical bone*

In the cortical bone the negative balance of the units of remodelling result in a thinning of the walls of the Havers' systems, which results in a widening of their channels. In the transversal histological cortex, this widening of the channels result in the presence of circular cavities, which give the bone tissue a porous aspect, which is why we talk of "cortical porosity".

On the other hand, in the osteones closest to the endosteum, the coincidence of thinning of the Havers' systems –due to the negative balance– with the greater movement of osteoclast –due to the raising of their activity– may result in a perforation in its wall, in such a way the Havers' channel makes contact with the tissue of the bone medulla. In such a case, this tissue enters towards the interior of the Havers' system, which ultimately suggests that the bone medulla gains space at the cost of what we could qualify as an endosteal recess. The result, logically, is a thinning of the cortex.

#### *c) Consequences common to trabecular and cortical bone: modification in the intrinsic properties of bone tissue*

The increase in turnover, in addition to the inconveniences indicated, has the impact of negatively modifying the intrinsic properties of bone material, due to what is thought to be the existence of an excessive quality of juvenile and immature bone<sup>41</sup>. The ideal properties of bone tissue are those corresponding to mature bone. The maturation of bone tissue involves different phenomena, of which should be mentioned the development of collagen bridges of certain characteristics, mineralisation carried out in two phases (primary and secondary mineralisation), with the hydroxyapatite crystals reaching sufficient size. The too rapid renewal of the bone does not allow the maturation of the collagen bridges, the secondary mineralisation, or the formation of hydroxyapatite crystals of the correct size.

On the other hand, the increase in turnover could have a beneficial effect, in the first place by avoiding the accumulation of microlesions due to fatigue, which tend to increase as the bone ages, and secondly due to the difficulty of their propagation, given the greatest heterogeneity in the mineralisation of the osteones that this implies (the oldest more mineralised, the youngest, less). However, the reduction in bone mass implied by the increase in turnover means that the usual load is, in relative terms, an overload, which should lead to a greater number of microlesions. This, along with the fact that the exact implications of



the microlesions is not well known, especially within physiological levels<sup>42</sup>, means that these comments should be considered as merely speculative.

#### d) Recapitulation

Therefore, the phenomena determining bone fragility as a consequence of an alteration in the functioning of the remodelling units characteristic of osteoporosis, are the following:

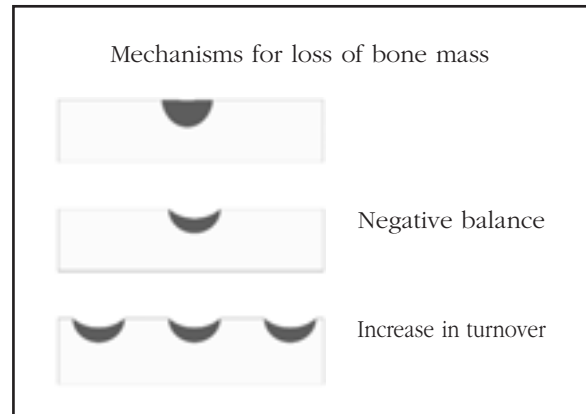
- Thinning of the trabeculae and of the cortex.
- Disappearance of part of the trabecular framework with their disconnection.
- Increase in the number of tension concentrators in the trabeculae.
- Cortical porosity.
- Immaturity of bone tissue.

### The consequences of the intermittent administration of PTH on the alterations in structure and bone quality characteristic of osteoporosis

We have indicated already that PTH administered continuously generally results in a reduction in bone mass, principally as a consequence of a stimulation in the activity of the osteoclasts, and therefore, bone resorption. However, administered intermittently it has a bone forming, also called anabolic, effect. The cellular mechanisms which drive this effect are dealt with later. Now we are going to focus on its repercussions on bone structure and bone mass<sup>43,44</sup>.

It is possible to distinguish two phases in the effect of PTH administered intermittently: the first, of some months duration, in which only the activity of the osteoblasts is increased, with the consequent bone forming effects, and the second, in which there is an increase in the activity of both the osteoblasts and the osteoclasts, in such a way that what ultimately happens is an increase in bone turnover with a positive balance, whose result, as we will see, is also bone forming. The increase in the osteoresorptive activity is detected some months after the start of the administration of the hormone. In the first phase, both the osteoblasts which are acting on the active units of bone remodelling and some of those which are found on quiescent surfaces (possibly the “coating” osteoblasts themselves)<sup>45</sup>, that is to say, those that have not undergone previous resorption: the osteoblasts of the external surface (periosteum), of the internal surface (endosteum), and of the trabecular surface which is not found in remodelling. In this final case, it appears that those osteoblasts found in proximity to the units of remodelling can be activated, while a possible alternative is that the active osteoblasts of those units themselves overflow their limits and occupy part of the surrounding bone. In whichever case, the possibility cannot be discounted that on the trabecular surface new bone is formed totally independent of the units of bone modelling, as has been argued<sup>45</sup> on the basis that the increase in volume of 35% at the end of the first year of administration of PTH could not be explained if it were only produced in

Figure 3. Negative balance of the unit of remodelling and increase in bone turnover as determining mechanisms in the loss of bone in osteoporosis



these units. The bone formed in sites previously subject to resorption (that is to say, in the units of remodelling) is sometimes called “remodelled bone” and that formed in places not subject to previous resorption (quiescent surfaces), as “modelled bone”.

The stimulus of the osteoblasts of the internal and external surfaces produces an increase in the thickness of the cortex, and therefore, in bone resistance. In particular, the subperiosteal deposition produces an increase in the external diameter of the bone, and it is worth noting that in this respect the mechanical efficacy given by a unit of bone tissue is higher the further its distance from the axis of the bone (greater module of inertia). Therefore, the bone tissue laid down below the periosteum is especially useful from a mechanical point of view. There are, however, doubts as to the exact extent of the subperiosteal apposition of the bone, and in whichever case it seems to be heterogeneous, in the sense that it is developed more in some bones than in others (probably more so in the tubular bones, above all if a mechanical stimulus is added, such as supporting weight, which works with PTH in its anabolic effect.

The units of remodelling which are active when the administration of PTH starts –more abundant in the trabecular bone– are essentially in a forming phase, since the action of the osteoclasts is very brief (some two to three weeks) in relation to the osteoblasts (several months). The stimulus of these osteoblasts puts the units of remodelling in positive balance, which causes an increase in the thickness of the osteone. As we have just indicated, some authors have said that the stimulator effect of PTH on the osteoblasts of the unit of remodelling extends to the surface osteoblasts which surround them, in such a way that the positive balance overflows the strict dimensions of the unit. These phenomena are responsible for a clear increase in trabecular bone volume.

The effect of PTH in stimulating the osteoblast starts to become noticeable in the second phase of its activity, which from this moment gives way to the birth of new and more numerous units of remodelling. That is to say, a phase of increased bone turnover begins. Given that the stimulator effect of the osteoblasts is maintained, this second phase is characterised by the combination of high turnover with positive balance. This results in the existence a great number of points in which bone is formed, which again, gives way to an increase in bone volume. For not very clear reasons, it increases also the number of trabeculae (it is not known if they are newly formed or results of tunnelling of thickened trabeculae). Trabecular connectivity also appears to be increased. The stimulator effect of the subperiosteal osteoblasts also appears to increase, so that the increase in cortical thickening continues. The increase in units of remodelling which characterise this phase, although favourable in the long term by increasing the number of places in which bone is formed by being in positive balance, could result in the fear of an initial transitory weakness in the skeleton by supposing places in which tension concentrations are established.

This is not confirmed in practice, which is probably due to the fact that the increase in bone volume makes the concentrations of tension at the points at which the units of remodelling are present, lower. However, we should point out that on occasions when the cortical bone near the endosteum, an increase in porosity can be observed on the administration of PTH, which definitely results in an increase in units of remodelling.

A theme frequently debated is if the increase in bone mass produced by the intermittent administration of PTH is contributed to more by remodelling bone, synthesised on earlier units of remodelling, or of modelling on previously quiescent surfaces. It seems beyond doubt that the former has much more importance. In any case, the relative importance varies from the former to the latter in the phases commented on. In the former, the modelled bone may be up to 30%; in the latter, much less: around 3-8%<sup>44</sup>. The reason for this is that in the latter case the number of units of remodelling is increased. In accordance with this, the principal bone forming effect of PTH takes place in the trabecular bone, which is where they are most abundant.

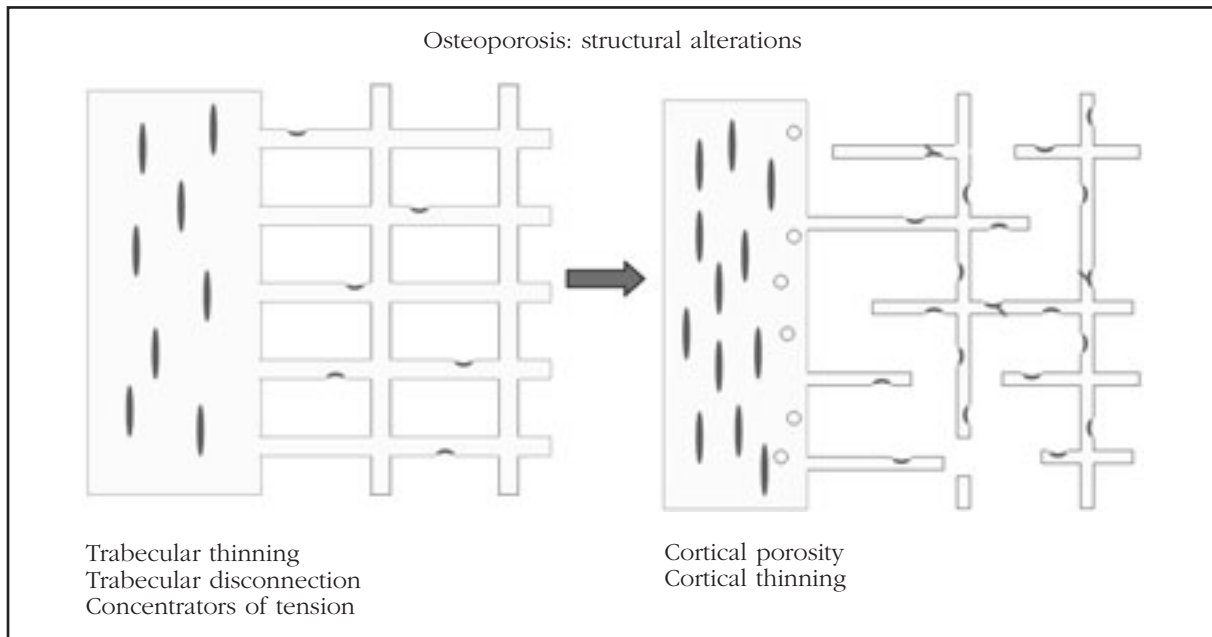
In summary, PTH administered intermittently modifies the bone structure in the sense of increasing the formation of bone on the trabecular, endocortical and periosteal surface, both in trabecular and cortical bone (Figure 4). All this results in an increase in bone resistance confirmed in biomechanical studies. The intensity of this effect may vary from one place to another, depending, among other factors, on the mechanical load which is established in the different locations. The increase in subendosteal porosity in some places, such as the radius, could raise a fear of a reduction in resistance, which however is not yet con-

firmed, most likely by the compensatory effect of the bone's subperiosteal growth.

During the first, solely bone forming, phase, an increase in markers for bone formation is detected in the blood. Later, to this increase in markers for formation is added an increase in those for resorption. The graphic representation (Figure 5) of this temporary behaviour of the two types of markers permits the observation of a space between the curves for each of them, before they finally join, once both are increased. This space corresponds to what we have just described as the first effect of the PTH, and was at one point called the "anabolic window". The term is a mistake, given that it may be interpreted as saying that only here does bone formation happen. This is clearly incorrect. In the second phase, although this stimulates resorption, formation predominates, given that there is a positive balance. What the resorption does is to mark the point of birth of the units of remodelling, and therefore the place in which the osteoblasts will act later. It should be noted that the osteoclasts contribute to the formation and activation of the osteoblasts (coupling) by means of various mechanisms (releasing substances from the destroyed bone, producing soluble factors which stimulate the osteoblasts, through membrane molecules such as the ephrins, etc.), some of which are stimulators of PTH (ephrins), which favour the positive balance of the units of remodelling. In fact, the lack of osteoclasts reduces markedly the effect of PTH. Some authors suggest that in order to activate this it is not necessary that the osteoclasts carry out their resorptive action, being present is sufficient, although they do not resorb bone.; in fact PTH itself could produce transitory action on the osteoclasts. However, others believe that in the absence of resorption the anabolic action of PTH cannot be fully expressed. The discrepancies in the results obtained when antiresorptives and PTH are administered in different patterns most likely have to do with these aspects, not yet sufficiently clarified.

The change in bone mass - defined by densitometry - shows a rapid increase in the first 6-12 months, becoming attenuated later. At one point it was thought that it would practically disappear after around two years, although a recent study of steroidal osteoporosis over three years confirmed a continuation of the increase in bone mass during the third year, although of lower intensity. The levels of markers, however, do appear to diminish progressively, as the image in Figure 5 indicates. The reason for this behaviour, and of this possible limitation in the bone forming effect of PTH after a certain period of intermittent administration, is not known. It is possible that once a certain bone mass is attained, a kind of mechanostatic mechanism makes difficult the later apposition of the bone. One cannot discount the phenomenon of cell desensitisation to hormones. It is also possible that, with the passing of time, in the unit of remodelling the resorptive activity increases compared to the forming.

Figure 4. Structural alterations in the bone determined by the negative balance of the units of bone remodeling and the increase in bone turnover



Before leaving this section it is worth giving some consideration to the quality of bone tissue formed under the action of PTH<sup>43,44</sup>. We refer here to the quality of the bone material, since that of bone as a whole –in which structural aspects are predominant– is clearly improved by PTH, as has been deduced from the results of biomechanical studies already quoted. The characteristics of the bone material are essentially determined by the fact that they are found in a situation subject to high turnover. On average we are dealing here with younger bone before treatment, with collagen in which there is a high proportion of divalent bridges. The osteones are frequently renewed before they experience secondary mineralisation, for which reason the overall bone mineralisation is lower. On the other hand, in being renewed more quickly, the accumulation of microlesions in the bone tissue ought to be less (although this matter has not yet been confirmed). This is, without doubt, beneficial, as it is the heterogeneity of the mineralisation of the osteones which makes difficult the propagation of these microlesions. On the other hand, the lower mineralisation and the lack of maturation of the collagen may be unfavourable by reducing resistance. It is difficult therefore, to foresee the final result of these changes on the intrinsic biomechanical characteristics of the bone material.

#### Action mechanism of PTH at a cellular level

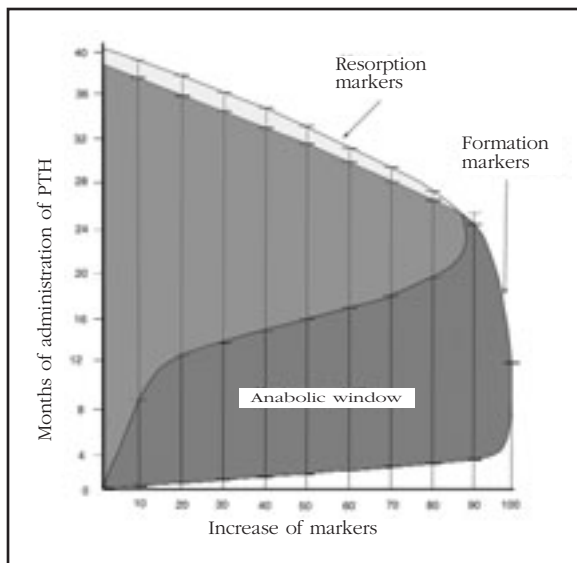
As we have seen, the aspect which defines the effect of the intermittent, as opposed to continuous administration of PTH, is osteoblastic stimulation. The hormone on the one hand increases the number of osteoblasts, and on the other, their activity. This is partly an indirect phenomenon, mediated

through the osteoclasts, and simply represents the consequence of the phenomenon of coupling, with a higher production by these cells than stimulatory factors of the osteoblasts. But at the margin, PTH exerts a direct effect by means of various mechanisms. For example, it increases the number of osteoblasts, stimulating their differentiation and inhibiting their apoptosis. It may also increase the proliferation of their precursors, although this effect is disputed. On the other hand, it stimulates the activity of the mature osteoblasts. Of the two effects, the first appears to be the most important by far, judging by the histomorphometric studies (greater increase in the surface of mineralisation than of the speed of mineral apposition). The reduction in apoptosis seems less significant in the periosteal bone than in the trabecular.

The action of PTH on the osteoblasts takes place through the receptor PTHR1, and its anabolic effects are principally mediated by the cAMP-PKA pathway<sup>46</sup>. It is possible that exogenous PTH, administered intermittently, may reproduce the effect of the endogenous PTHrP.

The end result of the action of PTH on the osteoblasts appears to be highly varied, and involve agents of different kinds<sup>47-49</sup>: osteoblast stimulatory factors, for which they have specific receptors, antagonists of the aforementioned ligands; certain receptors; various signalling pathways and transcription factors. Among the osteoblast stimulatory factors have been described some Wnt proteins, BMP2, the IGFs, FGF2, TGF $\beta$  –which would act in the form of autocrine or paracrine– as well as 1.25 (OH) $_2$ D, which, after being synthesised in the lower kidney stimulates the PTH, would be in the form of endocrine. At one point special impor-

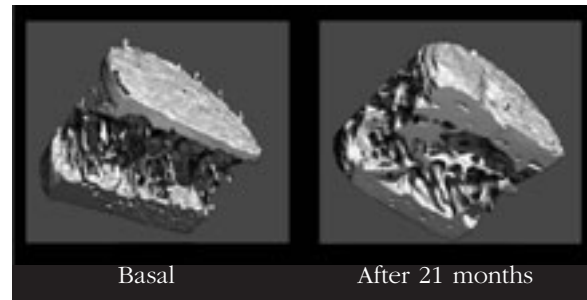
Figure 5. Temporal relationship between the changes in markers for formation and resorption induced by PTH, which led to the (inexact) concept of a therapeutic window



tance was given to IGF, proposing that in its absence PTH does not have an anabolic effect. Among the regulatory factors of these ligands, sclerostin should be mentioned, a substance produced by the osteocytes with an inhibitory effect on the action the Wnt proteins achieved by bonding to its receptor in the LRP5 component. The secretion of sclerostin by the osteocytes is slowed by PTH. PTH also suppresses other antagonists to the Wnt pathway such as DKK1 and SFRP-1. As receptors which can be modulated by the hormone, EGFR –whose ligands to these effects would be amphiregulin, RAGE– essentially in the spongy bone of the proximal femur, and the system of ephrins in the osteoblast, have been indicated. The intracellular agents (elements of the signalling pathways and transcription factors) which have been involved in the anabolic effect of PTH are also numerous: runx, osterix, ATF4 – stimulated by the hormone, PPAR $\gamma$  – all involved in osteoclast differentiation, or the protein Bad, with a proapoptotic effect, and which is deactivated by PTH. Apoptosis is a critical factor in the determination of the number of osteoblasts.

In summary, PTH performs its bone forming effect by stimulating the osteoblasts through multiple mechanisms. It is appropriate to talk of the “pleiotropic effects of PTH”. However, we do not know their details very well, nor do we know up to what point these mechanisms may be vicariant, or up to what point they are indispensable, being fundamental points of regulation. The absence of some of these factors block the bone forming effect of PTH, but the same does not happen with others. On the other hand, it appears that the effect of these various mechanisms varies from one place in the skeleton to another.

Figure 6. Changes induced in bone structure by teriparatide



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