Vitamin D metabolism in renal disease

Sir Michael Perrin Lecture 1991

Before exploring the repercussions of the failing kidney on the organism, one must first consider the role of the normal kidney. According to the Talmud Berakhot (c. 300 AD): 'The organs of the body were created to perform ten functions, among which it is the function of the kidney to furnish the human being with thought'. This view, although flattering to the nephrologist, is no longer shared by many. It is safe to state, though, that the kidney is a remarkably intelligent organ.

The purpose of my lecture is to demonstrate that the kidney plays a key role in the endocrine control of calcium metabolism, and to point out to what extent recent insights gained by the techniques of cellular and molecular biology have helped to clarify some of the problems relating to the faulty interplay of calcium regulating hormones in renal failure. Such insights have helped to devise more rational strategies for the prevention and treatment of renal bone disease.

In order to elucidate the pathogenesis of deranged calcium metabolism it is useful to examine the initial stages of renal failure, before confounding factors such as hyperphosphataemia and metabolic acidosis obscure the situation in the terminal stage of renal failure.

Evidence of secondary hyperparathyroidism in the early stages of renal failure is provided by the high immunoreactive parathyroid hormone (PTH) levels even in patients whose glomerular filtration rate was not less than 80 ml/min. Two lines of evidence showed that the actions of PTH on its target organs are also increased. One is the increased fractional urinary excretion of cyclic AMP, the second messenger of PTH. The other is an increased number of osteoclasts and a greater proportion of woven, ie newly formed poorly textured, osteoid. Both observations point to stimulation of target organs by PTH.

Today, the immunoradiometric assay for intact 1,84 PTH provides evidence that, even at a GFR between 60 and 90 ml/min, a sizeable proportion of patients already have elevated PTH levels. This finding raises the question of what signal triggers hyperparathyroidism.

To explain the secondary hyperparathyroidism of renal failure, it was proposed that, as the serum phosphate would rise, at least transiently, after meals in patients with decreased GFR, so the concentration of ionised calcium would decrease; the drop in calcium concentration was thought to stimulate the secretion of PTH which in turn would increase the renal clearance of phosphate, so returning serum phosphate concentration to normal but at the price of elevated PTH levels.

While there is indeed good evidence that a phosphate load raises serum PTH levels in advanced renal failure, several observations fail to support the hypothesis that in the very first stages of renal failure there is a 'trade-off' between homeostasis of serum phosphate and high PTH levels. We found no increase of serum phosphate, either fasting or in the postprandial state, in patients with early renal failure. Furthermore, even in the absence of PTH, ie after parathyroidectomy, subtotally nephrectomised animals were able to adapt renal tubular phosphate transport to changes in phosphate load. This illustrates that PTH is not indispensable for homeostatic adjustment of renal phosphate excretion.

Although the trade-off theory in its original form is no longer tenable, it may contain a grain of truth. I suggest that secondary hyperparathyroidism is a 'trade-off' not for homeostasis of phosphataemia but for maintaining normal plasma levels of the renal metabolite, $1,25(OH)_2$ vitamin D_3.

It used to be thought that the main stimulus for parathyroid gland activity was a fall of ionised calcium concentration in the extracellular milieu but recent research has shown that, at least in some systems, negative hormonal feedback via the renal metabolite $1,25(OH)_2$ vitamin D_3 is quantitatively a more important stimulus of parathyroid gland activity. When calcium was lowered in rats by intraperitoneal injection of phosphate, the concentration of messenger RNA (mRNA) for pre-pro-PTH, the precursor for PTH synthesis, was greater than in control animals. When $1,25(OH)_2D_3$ was administered, serum calcium did not change but mRNA decreased. When the stimulatory and the inhibitory signals were combined by simultaneous administration of phosphate and $1,25(OH)_2D_3$, the net result was diminution of the mRNA.

How does $1,25(OH)_2D_3$ act on the parathyroid gland? As shown schematically in Fig. 1, renal 1α-hydroxylase hydroxylates the precursor $25(OH)D_3$ to the renal metabolite $1,25(OH)_2D_3$. The kidney and the parathyroid gland are part of an integrated endocrine control system: $1,25(OH)_2D_3$ suppresses the parathyroid cells on three levels:

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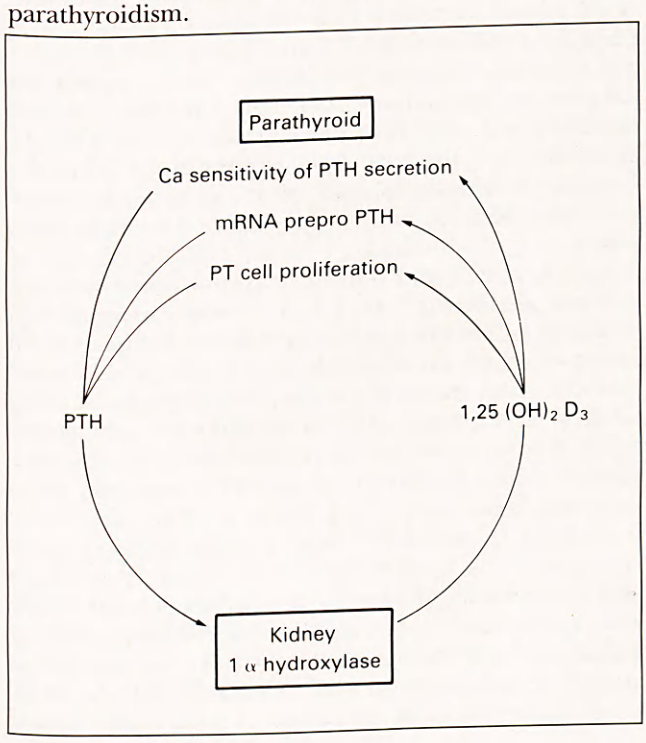
(1) It renders the parathyroid cell more sensitive to calcium, so that PTH secretion is suppressed by lower concentrations of ionised serum calcium (in other words the set point for PTH secretion is lowered.
(2) It inhibits the synthesis of RNA for pre-pro-PTH.
(3) It inhibits parathyroid cell proliferation.

In addition to the increase in the parathyroid cell synthesis of mRNA for pre-pro-PTH, recruitment in the number of parathyroid cells plays a major role in adaptation of the parathyroid gland to renal failure. This mechanism is called upon very early after parathyroid stimulation. Conversely, once the size of the parathyroid gland has increased by hyperplasia, parathyroid involution is a slow process. It has been estimated that the fractional reduction of volume is no more than 3–5% per year, and increased parathyroid weights are found even 10 years after successful renal transplantation.

Mechanisms controlling parathyroid cell proliferation

We found that, within six days after subtotal nephrectomy, parathyroid weight was doubled with no change in protein/DNA ratio, and showed that the enlargement resulted from parathyroid cell proliferation. The intraperitoneal administration of 20 pmols $1,25(OH)_2D_3$ six days after subtotal nephrectomy completely normalised the increased parathyroid cell proliferation.

Fig. 1. *The inhibitory actions of $1,25(OH)_2D_3$ on the parathyroid gland.* In the healthy kidney, dietary vitamin D_3 is converted to $1,25(OH)_2D_3$ by 1α-hydroxylase. The failing kidney makes inadequate amounts of $1,25(OH)_2D_3$ and this results in secondary hyperparathyroidism.



Reversal of parathyroid hyperplasia by apoptosis* is a slow process, and may not be speeded by $1,25(OH)_2D_3$. This provides a strong argument for trying to prevent parathyroid hyperplasia in patients with chronic renal failure by prophylactic administration of $1,25(OH)_2D_3$, and not delaying therapeutic intervention until florid hyperparathyroidism has supervened.

The important role of $1,25(OH)_2D_3$ in the control of parathyroid growth can be illustrated by cell culture studies. In parathyroid cell cultures, $1,25(OH)_2D_3$ lowered the rate of radiothymidine incorporation (Fig. 2a), and cell number (Fig. 2b). When cultured cells were stimulated by addition of fetal calf serum, the mRNA for the proto-oncogene-*myc* was increased, and was strongly inhibited by $1,25(OH)_2D_3$ (Fig. 2b).

How do these findings relate to the genesis of hyperparathyroidism of renal failure? When supranormal ionised calcium concentrations were maintained in nephrectomised dogs by administering calcium carbonate, PTH levels increased with time despite high levels of ionised calcium in serum, but when $1,25(OH)_2D_3$ was administered at the same time PTH levels remained normal.

If hyperparathyroidism is due to deficient renal production of $1,25(OH)_2D_3$, one would have expected to find low $1,25(OH)_2D_3$ levels in early renal failure. Yet paradoxically, both in humans with renal failure and in experimental animals, several authors have reported normal levels of $1,25(OH)_2D_3$. Three explanations have been put forward to account for elevated PTH levels despite $1,25(OH)_2D_3$ levels within the normal range.

(1) Sensing of $1,25(OH)_2D_3$ concentrations by the parathyroid may be lessened by a decrease in the number of $1,25(OH)_2D_3$ receptors on parathyroid cells.
(2) Levels of $1,25(OH)_2D_3$ in most patients may still be within the normal range although the average concentrations are already decreased.
(3) Levels of $1,25(OH)_2D_3$ may be kept within the normal range by raised PTH levels as a result of the feedback relationship between the two hormones.

We examined the interaction of PTH on the renal 1α-hydroxylase by assessing the maximum increase in circulating $1,25(OH)_2D_3$ concentration in response to an intravenous bolus of the aminoterminal 1,38 fragment of human PTH. As expected, the hPTH fragment raised serum calcium, diminished calciuria, reduced serum phosphate, and raised urinary cyclic

* Apoptosis: a gene-directed programme of cell death (review). *J R Coll Physicians* 1992;**26:**25–35.

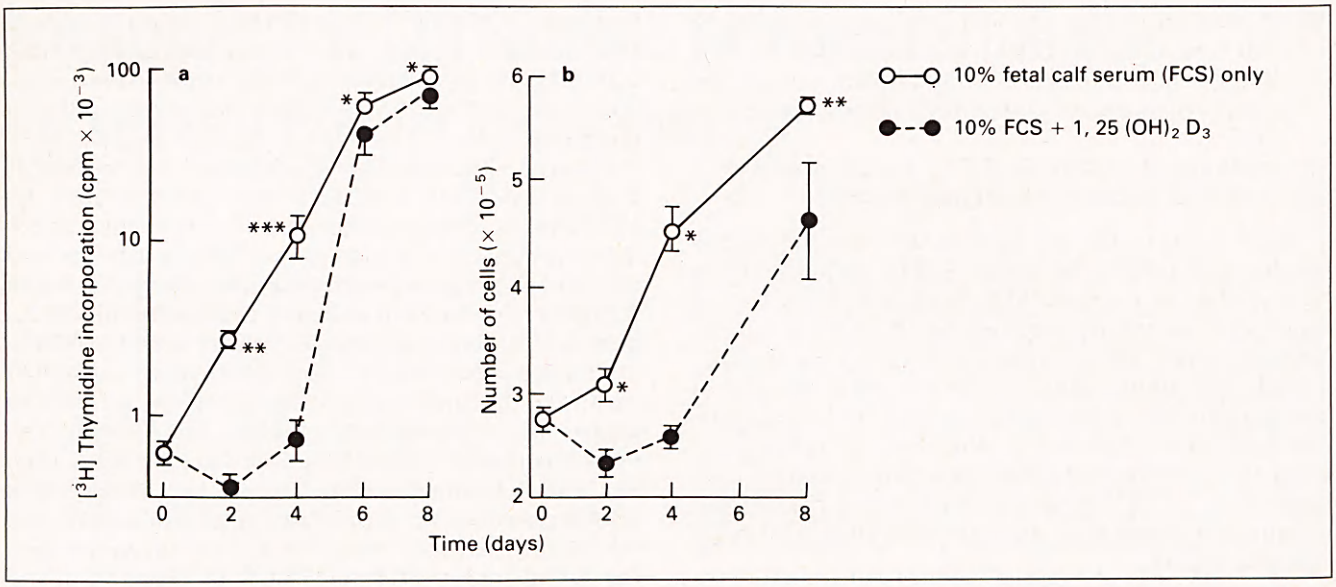


Fig. 2. *Influence of calcium and 1,25-dihydroxy vitamin D_3 on proliferation and proto-oncogene expression in primary cultures of bovine parathyroid cells.* With permission from the editor of *Endocrinology* (Kremer R *et al. Endocrinology* 1989;**125:**935–41.)

AMP in patients with incipient renal failure and in controls. The increased cyclic AMP excretion illustrates that renal tubular cells interact with PTH, thus excluding the possibility of generalised PTH resistance. Although patients had baseline $1,25(OH)_2D_3$ concentrations within the normal range, the increase in their $1,25(OH)_2D_3$ concentration was only 15.5%, as against 89.9% in controls.

What is the explanation? The increment in $1,25(OH)_2D_3$ was lowest in those patients who had the highest PTH levels. This suggests that in some patients renal 1α-hydroxylase activity is 'driven' by endogenous oversecretion of PTH to such an extent that little further increase is possible in response to exogenous PTH.

This raises an interesting problem. In other endocrine organs one has to remove nine tenths of the gland before hormonal secretion is exhausted and levels of the hormone in the circulation decrease: for instance, one must destroy more than 90% of pancreatic islets before insulin concentration decreases. But in early renal failure, apart from a diminished number of nephrons, there may be, in addition, abnormal regulation of the rate-limiting enzyme 1α-hydroxylase.

It is possible that the imbalance between phosphate load and renal excretory capacity for phosphate may cause an early decrease of $1,25(OH)_2D_3$ biosynthesis. Also, when the number of nephrons is reduced in the failing kidney, the phosphate concentration in a hypothetical subcellular compartment of renal proximal tubular cells might increase. This would be similar to what occurs in response to a phosphate load: the cells should behave as if the organism were threatened by phosphate overload, ie tubular phosphate reabsorption should diminish (thus explaining the tendency for low serum phosphate levels in early renal failure), and the synthesis of $1,25(OH)_2D_3$ should diminish (thus explaining the tendency for low serum calcium levels and hyperparathyroidism). If this hypothesis were confirmed, it would be rational to try to prevent hyperparathyroidism in incipient renal failure by dietary phosphate restriction. To test this possibility, we studied healthy subjects and patients with early renal failure who were first examined on their usual phosphate intake and then on 400 mg phosphate per day without changing the dietary calorie, sodium or calcium intake. Urinary phosphate excretion reached equilibrium by the third day, but plasma $1,25(OH)_2D_3$ concentrations did not change significantly. Thus the role of phosphate, if any, in the genesis of hyperparathyroidism of early renal failure remains uncertain.

In the past, renal bone disease has been a major clinical problem in the UK, but was uncommon in adults in other European countries or in the US. An unexpected explanation for this clinical puzzle came recently from the observation that anephric patients who were thought to be unable to synthesise $1,25(OH)_2D_3$, were free of osteomalacia. But the conclusion that $1,25(OH)_2D_3$ was therefore unrelated to osteomalacia may have been premature, since $1,25(OH)_2D_3$ may have been synthesised in tissues other than the kidney. Extrarenal synthesis occurs in uraemic animals. More importantly, human osteoblasts also synthesise $1,25(OH)_2D_3$ and local synthesis may build up high local concentrations. An important paracrine or autocrine role of $1,25(OH)_2D_3$ in bone cannot, therefore, be excluded. This may also explain

Fig. 3. *Madonna and child (A. Dürer).* Note the hypotonic abdominal musculature—probably due to rickets.

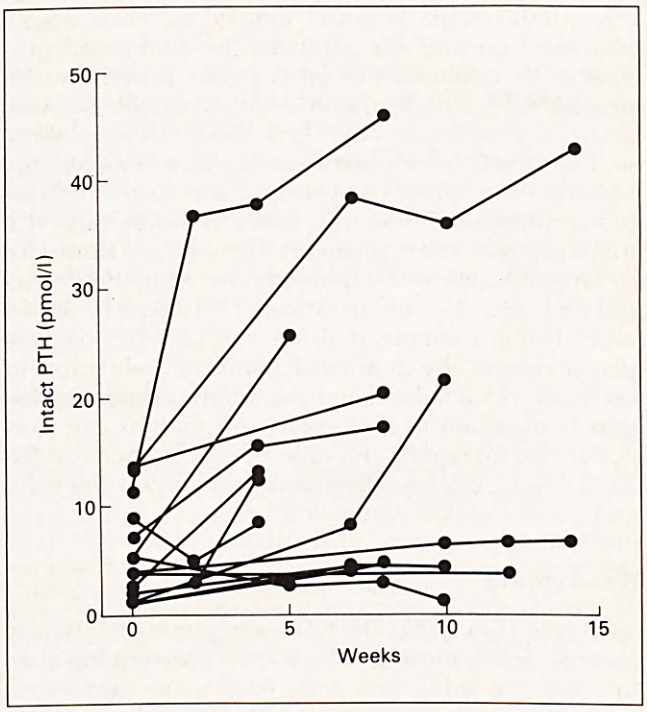


Fig. 4. *PTH secretion after withholding 1,25(OH)$_2$D$_3$ injections.* Suppressed PTH levels rose again after withholding the injections in eight patients but failed to rise in the other six patients.

a previously puzzling observation that in anuric patients, who are unable to synthesise the renal vitamin D metabolite, there is a relation between the presence of osteomalacia and 25(OH)D$_3$ levels. At the time, this could not be explained because 25(OH)D$_3$ was thought to be biologically inactive at normal concentrations. But, unlike renal synthesis, the extrarenal synthesis of 1,25(OH)$_2$D$_3$ depends on the concentration of its substrate 25(OH)D$_3$. Low 25(OH)D$_3$ levels may therefore lead to low local 1,25(OH)$_2$D$_3$ concentrations next to osteoblasts in bone. This suggests that vitamin D deficiency, which is not uncommon in these patients as a result of altered lifestyle, melanosis cutis, renal loss of protein-bound vitamin D metabolites in nephrotic proteinuria, etc, should be corrected by the administration of vitamin D.

Vitamin D deficiency

When vitamin D deficiency was still a common clinical problem, several facets in its clinical presentation were recognised which were unlikely to be due to disturbed homeostasis of serum calcium, for instance impaired macrophage function (which underlies the known propensity to infection) or a peculiar form of proximal myopathy.

It may be significant that artists in Northern countries, as illustrated by Albrecht Dürer's Dresdener Madonna (Fig. 3), commonly depicted the infant Christ with a rachitic 'frog belly' whereas artists from the sunlit Mediterranean shores consistently showed the Christ baby without such evidence of muscular hypotonia of rickets. Several plant species, when irradiated by ultraviolet light, produce vitamin D or even 1,25(OH)$_2$D$_3$. If nature has put vitamin D into a plant, it is logical to assume that this substance has a role to play, although by no stretch of the imagination can such a role be related to control of the calcium concentration in a (non-existent) extracellular fluid space. Vitamin D turns out to be a signal for cell differentiation; this is shown by the observation that a 1,25(OH)$_2$D$_3$ analogue stimulates root formation (rhizogenesis) by calcium-dependent mechanisms. This brings to mind Medawar's well-known statement that the evolution of hormones largely involves finding new uses for old hormones.

An action of vitamin D and the presence of receptors for vitamin D have been demonstrated in a number of organs or systems: endocrine cells, keratinocytes, muscle cells, neurons, and the immune system. The role of 1,25(OH)$_2$D$_3$ in immunoregulation is illustrated by the protective action on mice given

encephalitogenic doses of central nervous tissue. Improved survival was paralleled by diminished production of antibodies to myelin basic protein in the treated mice. Non-hypercalcaemic metabolites of vitamin D, eg calcitriol, can, like cyclosporin A, prolong skin allografts in rats across strong histocompatibility barriers, the effect of cyclosporin A and calcitriol being additive. These and other findings raise the question whether abnormalities other than the deranged calcium metabolism are also the consequence of low concentrations of $1,25(OH)_2D_3$ in uraemia. For example, deficiency of $1,25(OH)_2D_3$ may play a role in the abnormal immunomodulation of end-stage renal failure, and the administration of vitamin D metabolites to patients on dialysis can normalise the mitogenic response of T-cells, increase the T4/T8 ratio, increase the activity of natural killer cells, and normalise IL-2 production.

Renal growth

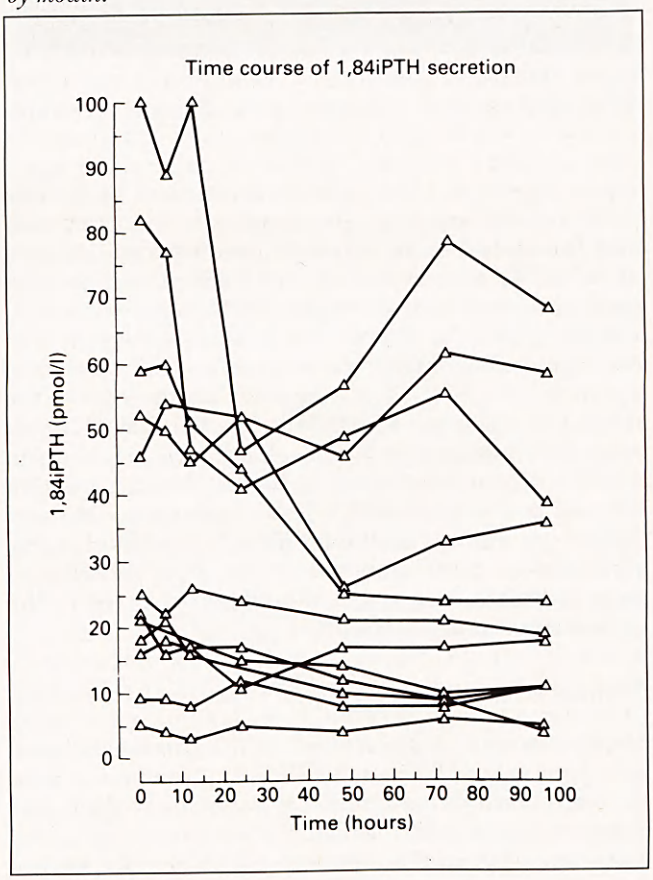
The fact that $1,25(OH)_2D_3$ is a phylogenetically ancient signal, moving cells from a proliferating state to a differentiating state, may have some unexpected consequences for the patient with renal failure. While investigating the effect of $1,25(OH)_2D_3$ on compensatory renal growth in rats two days after removing one of the kidneys, we found that physiological doses of $1,25(OH)_2D_3$ reduced the number of mitoses in the cortex and proximal tubules of the remaining kidney. We also found a dose-dependent reduction by $1,25(OH)_2D_3$ of radiothymidine incorporation and cell numbers in human mesangial cell cultures and in the proximal tubular OK cell line. It would thus appear that $1,25(OH)_2D_3$ interferes with renal growth, and that its administration may preserve renal function in glomerulosclerosis and slow the progression of renal failure. Indeed, a recent study suggests that the loss of renal function may be attenuated in patients treated with $1,25(OH)_2D_3$—a far cry from previous concerns that vitamin D metabolites might promote loss of renal function independent of calcaemia.

Treatment of secondary hyperparathyroidism

Recent progress in understanding the control of parathyroid cells by $1,25(OH)_2D_3$ has had considerable impact on our therapeutic strategies to treat the patient with symptomatic renal hyperparathyroidism. As an alternative to oral administration of $1,25(OH)_2D_3$, intravenous administration may be more effective in suppressing parathyroid overactivity: (a) it is possible to achieve higher peak concentrations and, by implication, higher occupation of $1,25(OH)_2D_3$ receptors; (b) at the same time it may reduce intestinal absorption of calcium as intestinal mucosal cells presumably 'see' less of the active vitamin D after injection; (c) bioavailability is greater with intravenous administration because this circumvents presystemic metabolism. However, high oral doses of $1,25(OH)_2D_3$ given intermittently did also reduce PTH levels in renal failure patients at the time of dialysis in our study. When $1,25(OH)_2D_3$ was withheld, intact PTH rose again in some patients, but this was by no means a uniform occurrence. In approximately one-half of the patients, PTH remained low after an eight-week course, (Fig. 4). Why is this important? We know that it is highly undesirable to oversuppress the parathyroid gland. When PTH levels are below the normal range, bone turnover slows down. Under normal circumstances, the skeleton has a finite rate of turnover and will adapt its architecture to carrying biomechanical loads. This is illustrated by the dramatic change in bone architecture after a fracture. Repair of microscopic injury causing microcallus formation also requires a finite level of bone turnover. To avoid oversuppression of PTH and the attendant suppression of bone turnover, it may be better only to administer $1,25(OH)_2D_3$ for as long as elevated PTH levels indicate the need for it.

One of our observations may help to improve the safety of vitamin D therapy: there were no episodes of hypercalcaemia while intact PTH levels were above the

Fig. 5. *In most patients PTH secretion remained suppressed for 96 hours or longer after a single dose of 2 mg $1,25(OH)_2D_3$ by mouth.*



normal range, probably because the skeleton, when exposed to high PTH concentrations while at the same time undergoing high turnover, retains calcium and thus reduces the risk of hypercalcaemia. It may not be necessary to administer $1,25(OH)_2D_3$ three times a week as recommended at present. We have shown that a bolus dose of $1,25(OH)_2D_3$ suppressed messenger RNA for pre-pro-PTH for up to 96 hours. The dose in these experiments corresponded to the estimated daily production of $1,25(OH)_2D_3$. Figure 5 shows the time course of intact PTH concentration in uraemic patients with various degrees of hyperparathyroidism after a single dose of 2 mg $1,25(OH)_2D_3$. Although in some patients PTH rose again after 48 hours, most patients showed a prolonged decrease of 1,84 PTH concentrations for 96 hours or longer. It is reasonable, therefore, to administer a high dose of $1,25(OH)_2D_3$ by mouth only once a week. Further, comparing the effects in uraemic rats of the same total dose of $1,25(OH)_2D_3$, given either as a bolus or as continuous infusion by osmotic minipump, we found that the bolus had the greater effect on parathyroid mRNA for pre-pro-PTH and on circulating aminoterminal PTH concentrations. Thus, knowledge of the mechanisms of control of the parathyroids by $1,25(OH)_2D_3$ through its receptor, a nuclear receptor of the steroid receptor superfamily, has helped to devise safer and more efficacious modalities of therapeutic intervention.

Envoi

The fascinating role of the kidney in the homeostasis of extracellular fluid, of which deranged hormonal control of calcium metabolism is just one example, was appropriately expounded in 1666 by Malpighi: 'In the past the kidneys had so variable a fortune as to be considered useless and unnecessary by some. More recently, they have been recognised as a marvellous structure, the function of which provides one of the most important in the human body'. To this I have nothing further to add.

Selected reading references

Reichel H, Drücke T, Ritz E. Disturbed calcium metabolism in renal failure. In: *Oxford textbook of clinical nephrology*, Vol 2. Cameron S, Davison AM, Grünfeld JP, Kerr D, Ritz E eds. Oxford: Oxford Medical Publications, 1992.

Reichel H, Deibert B, Schmidt-Gayk H, Ritz E. Calcium metabolism in early chronic renal failure: implications for the pathogenesis of hyperparathyroidism. *Nephrol Dial Transplant* 1991;**6:**162.

Delmez JA, Tindira C, Grooms P, *et al.* Parathyroid hormone suppression by intravenous 1,25-dihydroxyvitamin D_3. A role for increased sensitivity to calcium. *J Clin Invest* 1989;**83:**1349.

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