Vitamin D and Hyperparathyroidism

The Lumleian Lecture 1981

S.W. STANBURY, MD, FRCP

Professor of Medicine, University of Manchester

Albright defined primary hyperparathyroidism as 'a condition where more parathyroid hormone is manufactured than is needed', and secondary hyperparathyroidism as a state of affairs where 'more parathyroid hormone is needed than under normal circumstances' [1]. He recognised deprivation of calcium as the cause of secondary hyperparathyroidism and he speculated that clones of cells within secondarily hyperplastic glands might lose their normal secretory reactivity to calcium and thereby evolve to produce the functional state of primary hyperparathyroidism.

After Albright, it was tacitly assumed that the parathyroid glands in hypercalcaemic primary hyperparathyroidism were autonomous and unresponsive to changed concentrations of serum calcium; and that secondary hyperparathyroidism never progressed beyond the maintenance of a normal serum calcium to the production of actual hypercalcaemia. In the last decade it has been shown conclusively that neither of these assumptions is invariably valid; even the histological distinction between parathyroid hyperplasia and adenoma has become insecure.

This demonstration coincided fortuitously with explosive developments in the physiology of vitamin D. It is now known that the biological activity of vitamin D depends upon its prior metabolic conversion, first to 25-hydroxyvitamin D (25-(OH) D) in the liver, and subsequently, by the 1α-hydroxylation of 25-(OH)D in the kidney, to produce its effector molecule or hormonal form, 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) [2]. Most importantly, it was shown that parathyroid hormone is the principal trophic factor controlling the renal production of 1,25-$(OH)_2$D [3, 4]; and also that, in their action on bone, to elevate the serum calcium, each hormone 1,25-$(OH)_2$D and parathyroid hormone—requires the presence of the other for optimum effect [5, 6].

A host of recent animal experiments has identified many other factors, ionic and hormonal, capable of influencing the renal production of 1,25-$(OH)_2$D [7]; but, except for the effect of severe phosphorus depletion in increasing serum 1,25-$(OH)_2$D [8, 9], few have been proved to act independently of the parathyroid glands and few are of immediate clinical interest.

In healthy man, acutely induced changes of serum calcium or of dietary calcium produce reciprocal changes in serum 1,25-$(OH)_2$D [10, 11] and, since infused parathyroid extract can increase serum 1,25-$(OH)_2$D without significantly elevating the serum calcium [12], these effects are probably mediated by the accompanying changes of parathyroid secretion. The sustained inappropriate secretion of parathyroid hormone in primary hyperparathyroidism is associated with increased concentrations of serum 1,25-$(OH)_2$D [13, 14]; and there is now good evidence [15] that this is responsible for the well-documented increase of intestinal absorption of calcium in this disease [16, 17].

'Vitamin D deficiency' in its various forms (which would include the conditioned deficiency of 1,25-$(OH)_2$D in chronic renal failure [18]) is the commonest clinical cause of secondary hyperparathyroidism. Its study permits the clinician to make longitudinal observations of a nature qualitatively different from those of the designed, cross-sectional experiment. This provides at least the potential for uncovering relationships overlooked by the experimentalist. It is with some such clinical studies that the rest of this article is chiefly concerned.

The Osteomalacia of Vitamin D Deficiency

The reappearance in Britain of privational rickets and osteomalacia has provided the unexpected opportunity to apply modern techniques to the study of relationships between vitamin D and the parathyroid glands in man. It has been shown that these bone diseases can be healed and the accompanying secondary hyperparathyroidism reversed by oral treatment with 1,25-$(OH)_2D_3$ alone [19, 20] and, most significantly, that this can occur even when the serum 25-(OH)D remains very low or undetectable throughout the period of treatment [21]. This latter observation by Peacock is particularly important in that it almost conclusively disposes of the claim that 25-(OH)D itself [22, 23]—or other metabolites derived from it, such as 24,25-$(OH)_2$D [24], or 25,26-$(OH)_2$D [25]—play a direct role in facilitating the mineralisation of bone. It is not denied that pharmacological doses of 25-$(OH)D_3$ or of 24,25-$(OH)_2D_3$ can produce biological effects, both in a variety of experimental conditions and in man, but there is as yet no convincing evidence that these actions have a physiological counterpart. Consequently, these metabolites will receive no subsequent mention and the biological effects of vitamin D will be considered solely in terms of 1,25-$(OH)_2D_3$ and its established actions in facilitating the cellular uptake and transfer of calcium and phosphate ions.

The functional relevance of the various metabolites of vitamin D becomes evident when patients with hypocalcaemic nutritional osteomalacia are treated by appropriately small (200-500 iu or 5-12.5μg/day) replacement doses of the native vitamin. In untreated patients, the serum 25-(OH)D is very low and serum 1,25-$(OH)_2$D may be undetectable, but a single oral dose (450 iu) of vitamin D may restore a normal serum 1,25-$(OH)_2$D within 24 hours, without necessarily inducing a measurable increase in serum 25-(OH)D. Continued treatment, which, at this level of dosage, may not increase the serum 25-(OH)D above 15-20 nmol/litre rapidly produces grossly supranormal concentrations of serum 1,25-$(OH)_2$D (Fig. 1) that are maintained for weeks or months

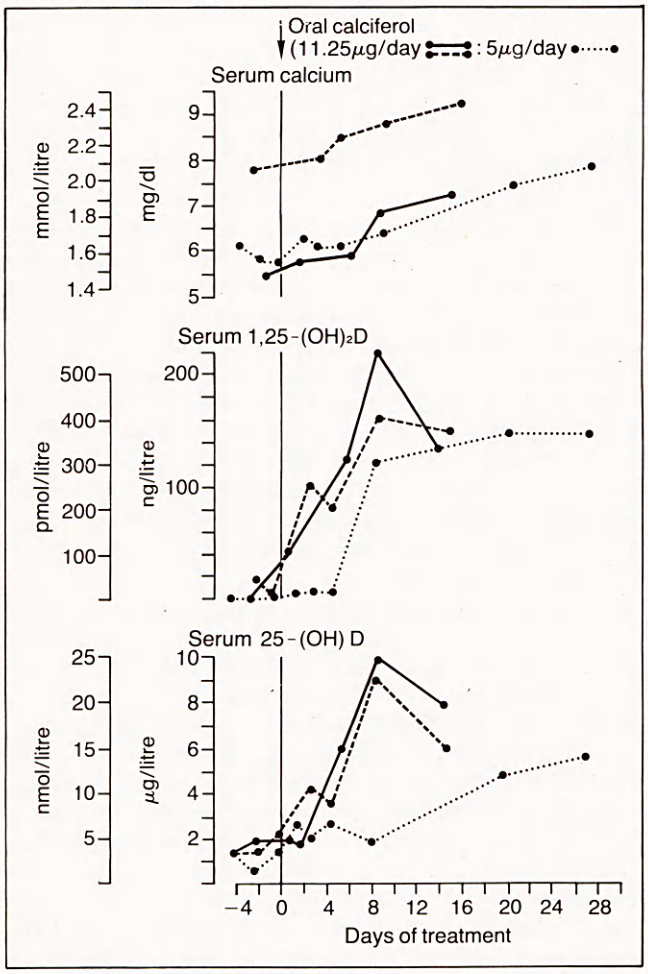


Fig. 1. *Biochemical changes following the institution of treatment with small doses of vitamin D in three patients with hypocalcaemic nutritional osteomalacia [28].*

[26—28]. A similar sustained elevation of the serum 1,25-$(OH)_2$D concentration occurs when the vitamin D deficiency is corrected physiologically by ultra-violet irradiation of the skin (Mawer, unpublished observations) rather than by oral therapy.

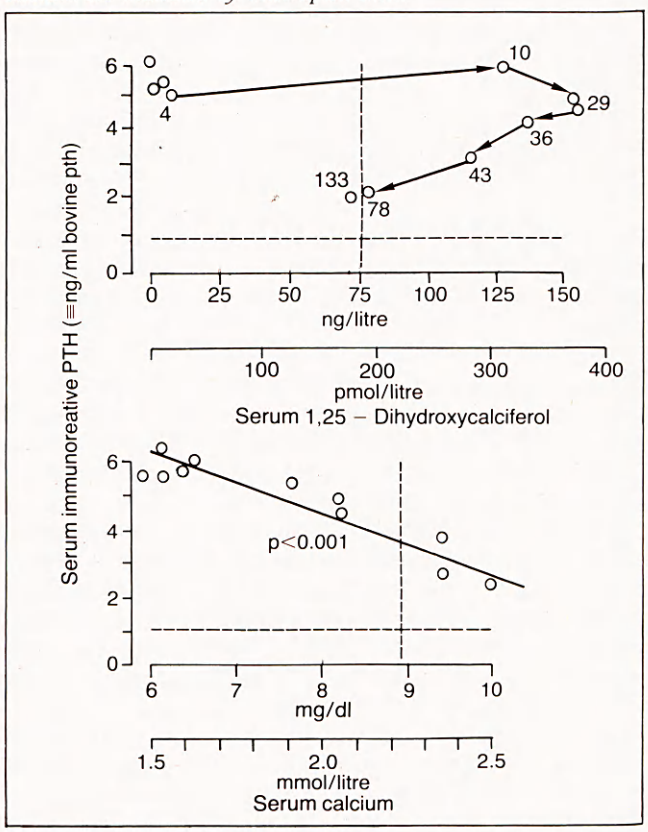
Thus, animal experiments which have been interpreted to imply that 1,25-$(OH)_2D_3$ alone fails to produce complete repair of the bone lesions of vitamin D deficiency [24, 29] have not reproduced the conditions that prevail during the physiological correction of the deficiency. Similarly, when oral dosage with 1,25-$(OH)_2D_3$ is reported as being less effective than 25-$(OH)D_3$ in repairing human osteomalacia [22], this is simply comparing the action of a small therapeutic dose of 1,25-$(OH)D_3$ with the greater quantity produced endogenously from the administered 25-$(OH)D_3$.

Just why supranormal amounts of 1,25-$(OH)_2$D should appear necessary for the healing of osteomalacia is not clear, although one is tempted to speculate that an increased turnover of bone might entail an increased osteoblastic requirement for the hormone. This interpretation would accord with the higher levels of serum 1,25-$(OH)_2$D found in growing children and adolescents and in pregnancy [30]—but not readily with the low serum 1,25-$(OH)_2$D and absence of osteomalacia in hyperthyroidism[31].

Several other points of importance emerge from these observations on the treatment of hypocalcaemic osteomalacia with vitamin D [28]. First, 24,25-$(OH)_2$D may be undetectable in the circulation until after 2-3 weeks of treatment, indicating that this metabolite is not required to initiate the mineralisation of osteomalacic bone. Secondly, the simultaneous presence of very high concentrations of both serum 1,25-$(OH)_2$D and serum iPTH

Fig. 2. *The relationship between the concentrations of serum iPTH and of serum 1,25-$(OH)_2$D* (above) *and serum calcium* (below) *during the treatment of a patient with nutritional osteomalacia with vitamin D.*

The numerals in the upper diagram refer to the days of continued vitamin D treatment. The upper normal limits of serum 1,25-$(OH)_2$D and serum iPTH and the lower limit of the serum calcium are indicated by interrupted lines.



(concentration in serum of immunoreactive parathyroid hormone) may not restore a normal serum calcium in these osteomalacic patients. Thirdly, elevated levels of serum 1,25-$(OH)_2D$ sustained for 2—3 weeks or longer appear not to act directly on the parathyroid glands to suppress their secretion (Fig. 2). Fourthly, after starting treatment, the serum calcium increases with the serum 1,25-$(OH)_2D$ (Fig. 1) and, during the first month of therapy, the increments in the two variables are positively correlated. This is the converse of the relationship obtaining in vitamin D replete individuals [10] and it is compatible with the concept that increased intestinal absorption of calcium is principally responsible for the restoration of normocalcaemia. Some of these points become relevant in patients with primary hyperparathyroidism whose disease is complicated by acquired vitamin D deficiency.

Effects of Vitamin D Deficiency in Primary Hyperparathyroidism

We have encountered about a dozen patients with primary (or tertiary) hyperparathyroidism and acquired vitamin D deficiency [32]. Vitamin D deficiency in this disease results in a lowering of the raised serum calcium, although not usually to reach the normal range, an increase of serum iPTH and a significant reduction in the urinary output of calcium (Fig. 3). The condition then resembles superficially the syndrome of hypocalciuric hypercalcaemia [33, 34] but differs in being associated with much higher levels of serum iPTH and with the presence of bone disease. As in simple vitamin D deficiency, treatment with small doses of vitamin D elevates the serum 1,25-$(OH)_2D$ to supranormal levels (Fig. 4), increases the serum calcium and reduces the serum iPTH.

The biochemical changes accompanying the development and correction of vitamin D deficiency in these patients evolve slowly over weeks or months, and throughout this prolonged period there is a highly significant inverse correlation between the serum iPTH and the serum calcium (Fig. 5). There may also be less close inverse correlations between serum iPTH and both the serum 25-(OH)D and serum 1,25-$(OH)_2D$ (Fig. 5); but, especially when coupled with the observations in privational osteomalacia (*see* Fig. 2) [28], the accumulated evidence indicates that vitamin D deficiency increases the static level of serum iPTH in primary hyperparathyroidism through its effect in reducing the serum calcium. Although specific cytoplasmic receptors for 1,25-$(OH)_2D_3$ have been demonstrated in parathyroid tissue [35, 36], these do not appear to be involved directly in this response to vitamin D deficiency. That statement contradicts a view expressed seven years ago [37] when we interpreted observations made on two patients only to imply that 'vitamin D' acted directly on the parathyroid glands to suppress their secretion. It does, however, accord with published studies showing that acutely induced changes of serum calcium in primary hyperparathyroidism produce reciprocal changes in serum iPTH [38-40]; and with the demonstration that surgically resected parathyroid tissue—whether 'hyperplastic' or 'adenomatous'—when cultured *in vitro* varies its hormonal secretion in response to changes in the concentrations of calcium in the incubating medium [41, 42].

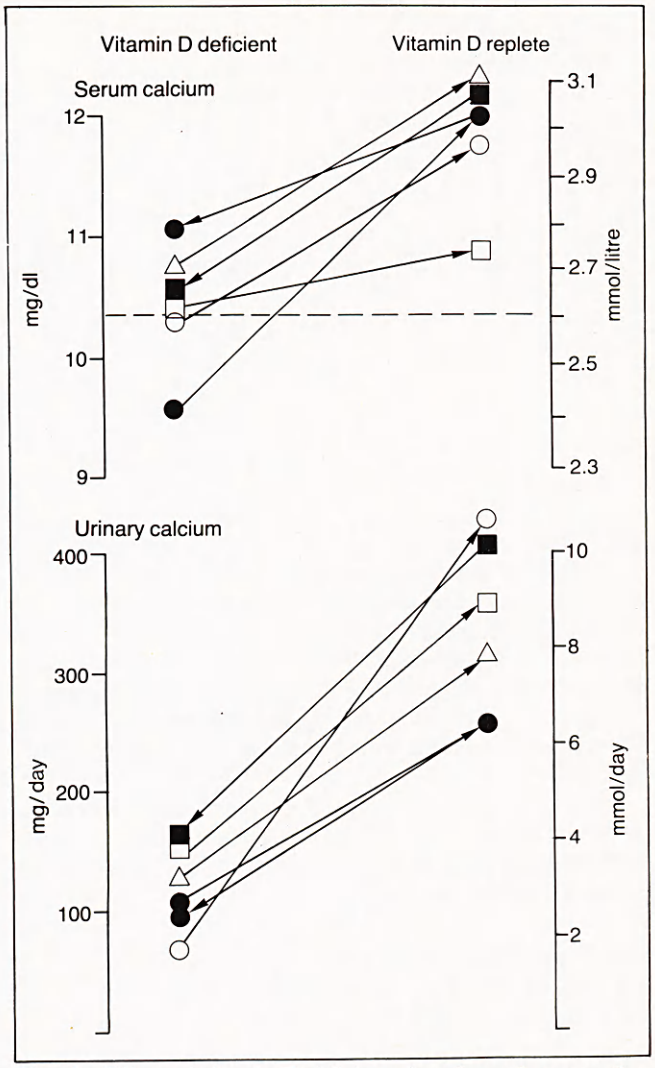


Fig. 3. *Effects of vitamin D deficiency in primary hyperparathyroidism. Data from six episodes of vitamin D deficiency in five patients are shown.*

The bone disease resulting from vitamin D deficiency in primary hyperparathyroidism can be indistinguishable from simple osteomalacia; but if the deficiency is prolonged and the serum iPTH becomes greatly elevated it can produce classical osteitis fibrosa, which is specifically reversed by correction of the deficiency [37]. This provides collateral evidence that the measured increase of serum iPTH with developing vitamin D deficiency in this disease provides a valid index of increased parathyroid secretion.

The acquisition of vitamin D deficiency in primary hyperparathyroidism can thus reversibly convert the disease from the type 'without bone disease' to the type 'with bone disease', or from Lloyd's [43] type 2 to type 1. The inappropriately secreting parathyroid tissue actually responds appropriately to the accompanying chronic changes of calcium metabolism. This is a paradox about which there is much speculation but for which there is no conclusive explanation.

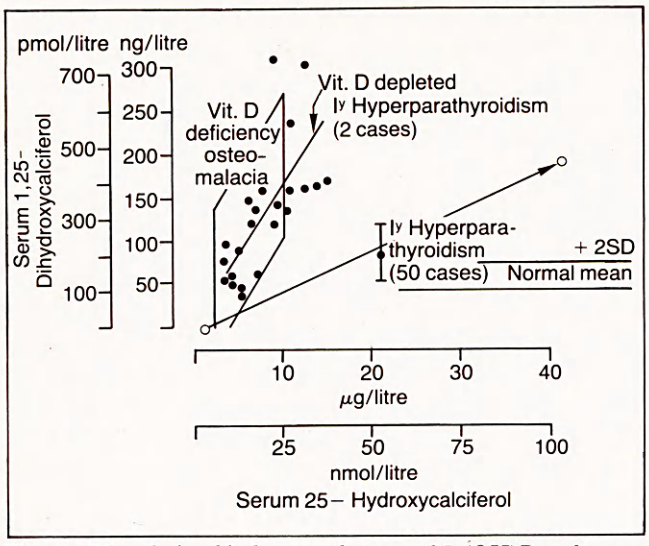


Fig. 4. *The relationship between the serum 25-(OH)D and serum 1,25-$(OH)_2D$ in the secondary hyperparathyroidism of vitamin D-deficiency osteomalacia and in primary hyperparathyroidism. To the left, the rhomboid outlines the confidence limits of the regression of serum 1,25-$(OH)_2D$ on serum 25-(OH)D for blood samples obtained during the first 50 days' treatment in the patients shown in Fig. 1. The open circles (○) connected by arrow illustrate the effect of treating nutritional osteomalacia with 25μg/day of 25-$(OH)D_3$.*

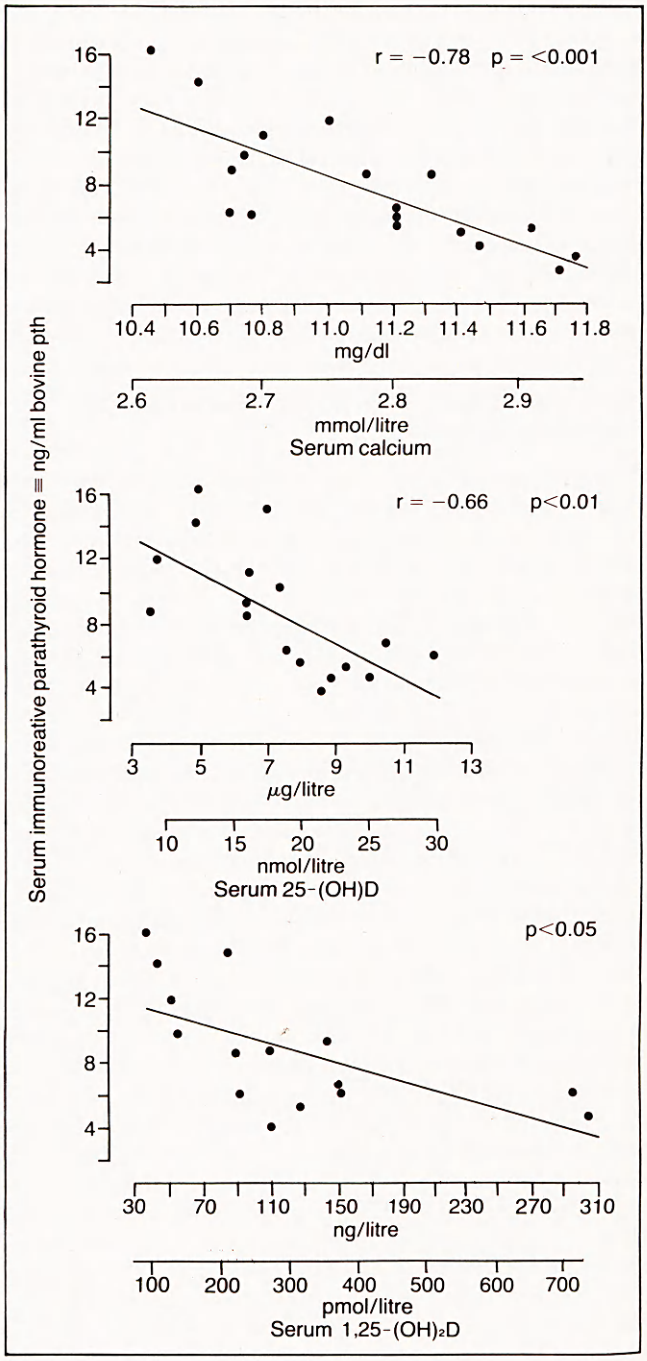
The small closed circles (●) and the regression line relate to two vitamin D depleted cases of primary hyperparathyroidism treated as in Fig. 1 [32]. Data for vitamin D replete primary hyperparathyroidism are taken from Broadus [15].

Relationship between Serum 1,25-$(OH)_2D$ and Serum 25-(OH)D in Secondary and Primary Hyperparathyroidism

Possibly the most interesting aspect of these clinical observations is the insight they provide into physiological control mechanisms. After starting the treatment of hypocalcaemic nutritional osteomalacia with small oral doses of vitamin D the concentrations of circulating 25-(OH)D and 1,25-$(OH)_2D$ increase together (*see* Fig. 1); during the first 4 to 6 weeks of such therapy there is a highly significant positive correlation between the concentrations of the two metabolites (*see* Fig. 4) [28].

In this state of secondary hyperparathyroidism the quantitative production of the hormonal metabolite appears to be a direct function of the circulating concentration of its precursor. The small doses of vitamin D (200-450 iu [5-11.25μg]/day) that we use customarily in treating nutritional osteomalacia may not increase the serum 25-(OH)D above 20-25 nmol/litre, but they can produce concentrations of serum 1,25-$(OH)_2D$ some four to five times the mean normal level [28]. Many physicians treat osteomalacia with much larger oral doses of vitamin D (3,000—5,000 iu [75-125μg]/day), which engender correspondingly higher concentrations of serum 25-(OH)D, but the scanty available evidence indicates that such dosage produces no greater concentrations of serum 1,25-$(OH)_2D$ [27]. We find that a daily oral dose of 25μg (≡6,000 iu) of 25-$(OH)D_3$ given to a patient with

Fig. 5. *The correlations of serum iPTH in a patient with primary hyperparathyroidism, during the correction and subsequent recurrence of vitamin D deficiency, with* (top) *the serum calcium,* (middle) *serum 25-(OH)D and* (bottom) *serum 1,25-$(OH)_2D$.*



osteomalacia may rapidly elevate the serum 25-(OH)D to 100 nmol/litre or more but the maximum concentrations of serum 1,25-$(OH)_2D$ attained are no higher than result from the much smaller doses of vitamin D itself (*see* Fig. 4) (unpublished observations).

Thus, early in the treatment of osteomalacia, the quantitative production of 1,25-$(OH)_2D$ is determined by the concentration of its precursor in the serum but there appears to be some mechanism that sets a limit to the

proportionate formation of 1,25-$(OH)_2$D when the provision of 25-(OH)D exceeds the physiological level. Later in treatment, as the osteomalacia heals, other mechanisms come into operation to restore the serum 1,25-$(OH)_2$D to normal levels [28].

In patients with primary hyperparathyroidism who are depleted of vitamin D, whether or not this has caused a complicating osteomalacia, small oral doses of vitamin D again produce proportionate increases of both serum 25-(OH)D and 1,25-$(OH)_2$D—the relationship being indistinguishable from that seen in the secondary hyperparathyroidism of simple nutritional osteomalacia (*see* Fig. 4). Yet, in the generality of vitamin D-replete patients with primary hyperparathyroidism, who have very much higher levels of serum 25-(OH)D, the mean serum 1,25-$(OH)_2$D is about two standard deviations above the normal mean (*see* Fig. 4) [13—15]. It is exceptional to encounter individual values more than 2.5 to 3 times the normal mean, and the higher levels encountered in healing osteomalacia are not seen. Since such vitamin D-replete patients have a sustained inappropriate, and often increased, secretion of parathyroid hormone and are also usually hypophosphataemic, some other factor must be restraining the proportionate production of 1,25-$(OH)_2$D with increase of serum 25-(OH)D.

Secondary hyperparathyroidism also occurs in association with rickets, and the biochemical features of vitamin D deficiency in the syndrome of 'pseudodeficiency' or 'vitamin D-dependent' rickets [44, 45]. In its classical form (type 1) there is probably a specific impairment of the renal production of 1,25-$(OH)_2$D [46, 47] and the condition is completely remediable by either pharmacological dosage of vitamin D [48] or microgram replacement doses of 1,25-$(OH)_2D_3$ itself [19, 49]. More recently, variants of this clinical syndrome (called type 2) have been described in which the renal mechanisms of 1α-hydroxylation are intact, and in which the biological features of 'vitamin D deficiency' persist despite the presence in the circulation of supranormal concentrations of 1,25-$(OH)_2$D and other hydroxylated metabolites of vitamin D [50-56]. It seems obvious from the published case reports that the term 'type 2 vitamin D-dependency' encompasses several distinct entities but, in one recessively inherited phenotype associated with alopecia totalis, therapeutic unresponsiveness has persisted even when treatment with enormous doses of vitamin D or 25-$(OH)D_3$ has induced concentrations of serum 1,25-$(OH)_2$D 50-100 times the normal mean value (Fig. 6) [52, 55].

The reported cases of type 2 vitamin D-dependency thus reflect different degrees of refractoriness of target organs to 1,25-$(OH)_2$D and probably a defect (or several phenotypically different defects) of its tissue receptors. In the context of the present discussion, the crucial point is that the concentration of 1,25-$(OH)_2$D in serum appears to increase with the serum 25-(OH)D—without the constraint evident in the other conditions discussed—to attain phenomenally high levels. When plotted on a log-log scale, data from the published cases of type 2 vitamin D-dependency, and from our own cases of nutritional

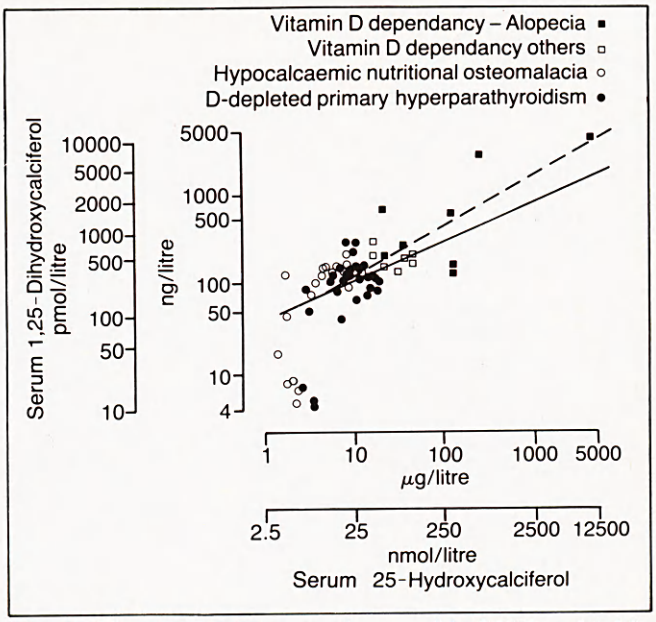


Fig. 6. *The relationship between the serum 25-(OH)D and 1,25-$(OH)_2$D in different types of hyperparathyroidism.*

Type 2 vitamin D dependency, data from published reports [50-56]; other data personal [28, 32]. Log-log scale.

The uninterrupted line is the regression fitted to the complete data ($p < 0.001$); the broken lines relates to type 2 vitamin D dependency only ($p < 0.01$).

osteomalacia and vitamin D depleted primary hyperparathyroidism fit to a single, highly significant regression of serum 1,25-$(OH)_2$D on serum 25-(OH)D (Fig. 6). In other words, by the appropriate selection of patients with hyperparathyroidism, one can demonstrate an apparent responsiveness of renal 1α-hydroxylation to an at least 2,000 times increase of serum 25-(OH)D.

In vitamin D dependency, the degree of hypocalcaemia, hypophosphataemia, secondary hyperparathyroidism and defective osseous mineralisation is not greater than in simple nutritional osteomalacia. It is therefore unlikely to be any of these factors that permits the unbridled accumulation of 1,25-$(OH)_2$D in the circulation with increase of serum 25-(OH)D. The answer may lie in the recent demonstration of a specific cytoplasmic receptor for 1,25-$(OH)_2D_3$ in renal tubular cells [57], with a defective responsiveness of this receptor in type 2 vitamin D-dependency preventing the so-called 'self-inhibition' of 1,25-$(OH)_2$D production [58—60]. The precise mechanism by which 1,25-$(OH)_2D_3$ produces inhibition of renal 1α-hydroxylase activity remains to be elucidated. As studied *in vitro* the process appears to involve a nuclear action with transcriptional protein synthesis in the renal cell [58, 59] and probably also the renal cellular uptake of calcium [58, 60].

If the factor determining 'self-inhibition' of 1,25-$(OH)_2$D production *in vivo* were the 1,25-$(OH)_2$D-mediated renal cellular uptake of calcium and the attainment of a critical calcium content in some intracellular compartment, the process might well be influenced by the calcium concentration of the extracellular fluid—as found in *in vitro* experiments [58, 60]. Thus self-inhibition might

be achieved less readily in the hypocalcaemic secondary hyperparathyroidism of nutritional osteomalacia than in hypercalcaemic primary hyperparathyroidism, thus explaining the higher concentrations of serum 1,25-$(OH)_2D$ attained in the former condition. Partial or complete failure of this inhibitory mechanism in type 2 vitamin D-dependency would have the functional effect of extending the range of concentrations of serum 25-(OH)D through which it determines the production of 1,25-$(OH)_2D$ (Fig. 6), and so account for the extremely high concentrations of serum 1,25-$(OH)_2D$ produced by vitamin D therapy in this syndrome.

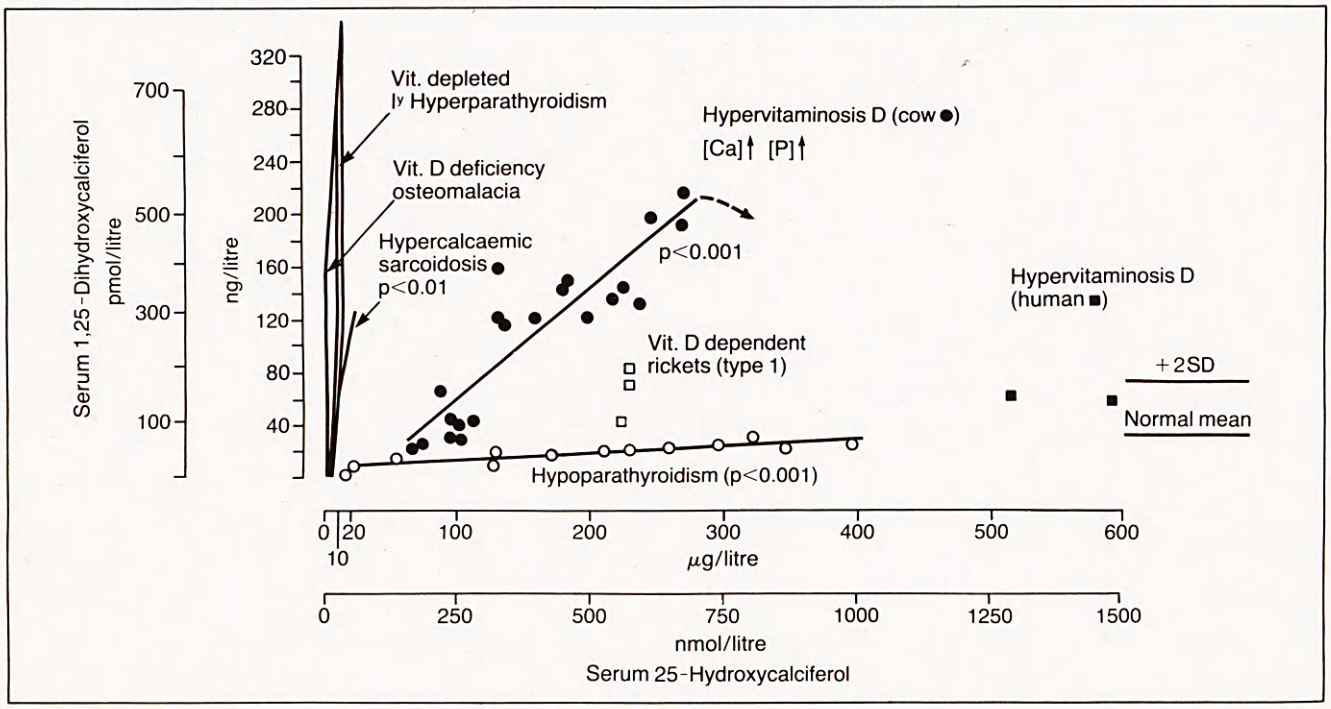
There is no direct evidence that the process of 'self-inhibition' functions as a physiological control mechanism when the serum 1,25-$(OH)_2D$ is within the range of normal. The observations cited suggest that it could become functionally operative in states of clinical hyperparathyroidism when the therapeutic provision of 25-(OH)D exceeds the physiological. The phenomena described are not explicable in terms of the other, conventionally accepted, control mechanisms.

The Ubiquity of the Relationship between Serum 25-(OH)D and Serum 1,25-$(OH)_2D$

Once aware of the relationship, it becomes possible to detect many other instances of an apparent dependence of serum 1,25-$(OH)_2D$ on the prevailing concentration of its precursor, serum 25-(OH)D (Fig. 7). In 13 patients with hypoparathyroidism, Lund *et al.* [64] demonstrated a highly significant positive correlation between the concentrations of the two metabolites. Low-normal values of serum 1,25-$(OH)_2D$ were attained when the serum 25-(OH)D was increased to 750-1,000 nmol/litre by vitamin D treatment; very low concentrations ($\leqslant$24 pmol/litre) were seen only in untreated patients. Thus, the functional significance of 1,25-$(OH)_2D$ deficiency in hypoparathyroidism (and probably also in pseudohypoparathyroidism) can be assessed adequately only in patients untreated with pharmacological doses of vitamin D or 25-$(OH)D_3$. The normal levels of serum 1,25-$(OH)_2D$ found [62] in patients with hypercalcaemic vitamin D intoxication—and presumed secondary hypoparathyroidism—fit well with the relationship demonstrated in primary hypoparathyroidism (Fig. 7). In patients with untreated type 1 vitamin D-dependent rickets 1,25-$(OH)_2D$ may be undetectable in the serum; but, despite the defective renal 1α-hydroxylase, normal concentrations can be attained when the serum 25-(OH)D exceeds 500 nmol/litre (Fig. 7) [52]. We have seen a similar increase of serum 1,25-$(OH)_2D$ with therapeutically induced increases of serum 25-(OH)D in patients with advanced renal impairment (creatinine clearance (C_{Cr}), 15-20 ml/min) (Mawer and Stanbury, unpublished) and the same phenomenon has been documented [65] in end-stage renal failure (C_{Cr} < 5 ml/min).

Two other instances of this relationship between the serum 1,25-$(OH)_2D$ and its precursor are of particular interest and importance—hypercalcaemic sarcoidosis and hypervitaminosis D in the cow. The inappropriate formation of 1,25-$(OH)_2D$ in hypercalcaemic sarcoidosis was probably first demonstrated in our own laboratory [66] but the observations of Bell and his collaborators have reasonably established the role of elevated levels of serum 1,25-$(OH)_2D$ in producing the abnormalities of calcium metabolism in this disease [67, 68]. In a single patient

Fig. 7. *Relationships between the serum 25-(OH)D and serum 1,25-$(OH)_2D$ in miscellaneous clinical states.*
Hypoparathyroidism [61]; human vitamin D intoxication [62]; bovine vitamin D intoxication [63]; type 1 vitamin D dependency [52]; sarcoidoisis [64]; nutritional osteomalacia and primary hyperparathyroidism [Fig. 4].



with sarcoidosis, O'Riordan and colleagues [64] showed that the development of hypercalcaemia after exposure to summer sunshine was associated with supranormal concentrations of serum 1,25-$(OH)_2D$. From their data it can be shown clearly that the serum 1,25-$(OH)_2D$ increased with the concentration of its precursor in a relationship quantitatively similar to that in healing osteomalacia (*see* Fig. 7). Similarly, in 'mature' cows treated with enormous and intoxicating doses of vitamin D, serum 25-(OH)D and serum 1,25-$(OH)_2D$ increased in parallel (*see* Fig. 7), despite rapidly developing hypercalcaemia and hyperphosphataemia [63].

It has become virtually accepted dogma that, whereas the concentration of serum 25-$(OH)D_3$ is a function of the delivery of cholecalciferol to the liver, the renal production of 1,25-$(OH)_2D_3$ is finely regulated independently of the quantitative availability of its precursor. In discussing the regulation of production of 1,25-$(OH)_2D_3$, Fraser [7] has emphasised the critical importance of access of the precursor, 25-$(OH)D_3$, to the 1α-hydroxylase on the inner side of the mitochondrial membrane in the renal tubular cells. He has speculated that regulatory factors, such as parathyroid hormone, might facilitate such access by changing the physical properties of the mitochondria or by influencing the production of a labile protein responsible for transporting 25-$(OH)D_3$ to the mitochondrial enzyme.

The examples cited indicate that the serum 25-(OH)D can be an important determinant of the serum 1,25-$(OH)_2D$ in human pathological states. Extrapolating simplistically from Fraser's concept, one might postulate that states of hyperparathyroidism in man 'facilitate' access to the renal 1α-hydroxylase, so that normal or increased circulating concentrations of 1,25-$(OH)_2D$ can be attained even when the serum 25-(OH)D is extremely low (5-15 nmol/litre, *see* Fig. 1). In the absence of such facilitation, in hypoparathyroidism, the mass action of excess 25-(OH)D is required to establish a 'normal' serum 1,25-$(OH)_2D$ (*see* Fig. 7). But in hypercalcaemic sarcoidosis and the vitamin D intoxicated cow, serum 1,25-$(OH)_2D$ increases with the concentration of its precursor to reach supranormal levels, in the absence of the recognised trophic and ionic factors generally considered to increase 1,25-$(OH)_2D$ production. (The published bovine data [63] do not, however, provide evidence of the state of parathyroid function in the treated animals.) Conceivably, these latter phenomena reflect respectively an acquired abnormality and an innate difference of the renal tubular cell mitochondria that render the 1α-hydroxylase more accessible to its substrate.

This evidence of an apparently causal relationship between the concentrations of serum 25-(OH)D and serum 1,25-$(OH)_2D$ in disease raises the question of whether there might be degrees of a similar relationship in healthy man. Gray and his colleagues [69] report relative stability of the serum 1,25-$(OH)_2D$ when measured in the individual over a period of 1-3 years, but their data show that its concentration in particular subjects could vary by as much as 30 per cent. It is well known that there is a seasonal variation in the absorption of dietary calcium, with higher values in the summer months [70, 71]; and there is a similar seasonal variation in the urinary output of calcium [72]. These latter changes echo, although they do not precisely coincide with, the described seasonal variations in serum 25-(OH)D ; but it is not known if there is a parallel seasonal variation in serum 1,25-$(OH)_2D$. With Dr E. Sommer of Hamburg, Dr E. B. Mawer has been measuring the changes in vitamin metabolites in the blood of patients receiving exposure to ultra-violet radiation as treatment for psoriasis. These observations are far from complete but they indicate that the induced increase of serum 25-(OH)D can be associated with an increase of serum 1,25-$(OH)_2D$, within the range of its normal variation among healthy individuals.

Thus, although not recognised by biochemists as a significant and important controlling influence, it seems likely that a degree of dependence of the quantitative production of 1,25-$(OH)_2D$ on the circulating concentration of its precursor is of universal occurrence in man. In otherwise healthy individuals, this relationship may attain functional significance only when the provision of vitamin D is restricted.

Adaptive Mechanisms in Vitamin D Depletion

It is generally agreed that privational osteomalacia usually develops only when the serum 25-(OH)D is less than 10 nmol/litre; but there are documented instances of bone disease in association with concentrations of serum 25-(OH)D of 15-20 nmol/litre and others in which concentrations of 5 nmol/litre or less show neither clinical, biochemical nor histological evidence of bone disease [73]. This proved absence of osteomalacia in individuals with negligible concentrations of serum 25-(OH)D has not been explained, nor has it been studied systematically. Its occurrence further reinforces one's view that 25-(OH)D has no direct role in the mineralisation of bone; and, since the level of serum 25-(OH)D is the principal determinant of the concentrations of serum 24,25-$(OH)_2D$ and 25,26-$(OH)_2D$ [74], it is equally unlikely that either of these metabolites serves that function.

In a recently undertaken survey of 262 immigrant Asian children and adults [75], we encountered 39 adults without clinical evidence of bone disease in whom the serum 25-(OH)D was less than 12.5 nmol/litre. In this sub-group the mean serum calcium, although normal (2.34, SD 0.09 mmol/litre), was significantly lower than the mean value (2.43, SD 0.08 mmol/litre) in 40 unquestionably vitamin D replete Caucasian control subjects, but only in eight individuals (16 per cent) was there homeostatic failure, with the serum calcium below the normal range (Fig. 8). The mean serum inorganic phosphorus concentration was also less than in controls (Fig. 8). In 30 of these vitamin D-depleted individuals, with concentrations of serum calcium within the normal range, the mean calcium-creatinine ratio in a casual urine specimen was significantly lower than in 26 control subjects with matched concentrations of serum calcium (Fig. 9).

This indirect evidence of secondary hyperparathyroidism in these Asian adults was confirmed more directly by the finding of a significantly higher mean concentra-

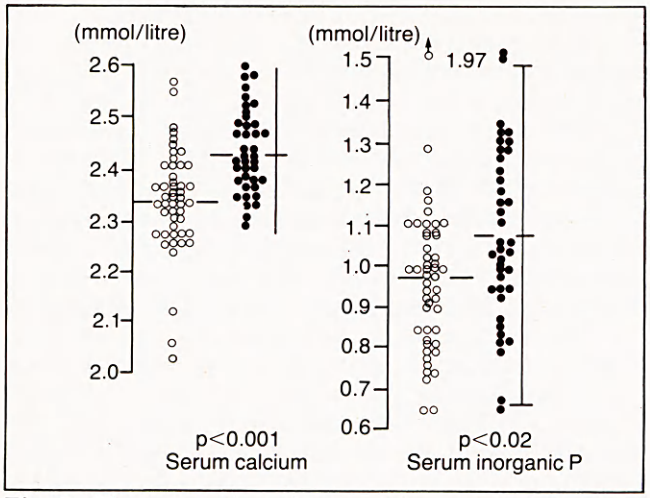


Fig. 8. *The concentrations of serum calcium and serum inorganic phosphorus in 39 clinically healthy Asians (○) with vitamin D depletion (serum 25-(OH)D < 5 μg/litre) compared with values in 40 vitamin D replete control subjects (●).*

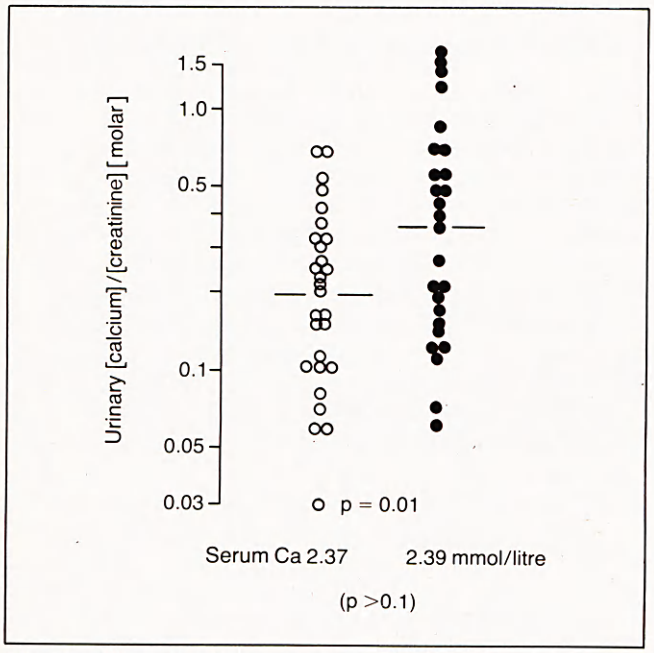


Fig. 9. *The ratio of concentrations of calcium to creatinine in casual urine specimens from 30 normocalcaemic, vitamin D depleted (serum 25-(OH)D < 5 μg/litre) subjects (○) compared with values in 26 vitamin D replete control subjects (●) with matched concentrations of serum calcium.*

tion of serum iPTH than in control subjects. At this stage of asymptomatic vitamin D depletion, a degree of secondary hyperparathyroidism appears to be required to maintain the serum calcium within the normal range in the face of a reduced throughput of calcium into the urine. This would imply that the first consequence of an inadequacy of vitamin D is probably a state of 'normocalcaemic secondary hyperparathyroidism'.

In the light of previous discussion, it seems eminently logical to postulate that the extremely low levels of serum 25-(OH)D in these individuals would inevitably result in a reduced serum 1,25-$(OH)_2$D. This could cause lowering of the serum calcium by impairing the intestinal absorption of calcium or by reducing the release of calcium from bone—or by both mechanisms. The reactive secondary hyperparathyroidism would increase the serum calcium by increasing the renal tubular reabsorption of calcium and probably also by tending to correct its original cause, an inadequacy of 1,25-$(OH)_2$D. We have seen in clinically overt vitamin D deficiency that secondary hyperparathyroidism could facilitate the establishment of normal or even increased levels of serum 1,25-$(OH)_2$D when the concentration of serum 25-(OH)D was as low as 5-10 nmol/litre (*see* Fig. 1). Thus, with developing vitamin D depletion, the most important function of secondary hyperparathyroidism could be to sustain the level of serum 1,25-$(OH)_2$D in the face of a diminishing serum 25-(OH)D . The process would continue until the serum 25-(OH)D was too low to permit significant access of precursor to the activated enzyme, when the serum 1,25-$(OH)_2$D would fall progressively towards undetectable levels [27, 28].

Among an asymptomatic vitamin D depleted population one would expect to find individuals in whom the adaptive process is effective, with normal or possibly even increased levels of serum 1,25-$(OH)_2$D and no osteomalacia; and others in whom the process has failed, the serum 1,25-$(OH)_2$D being subnormal and osteomalacia present or threatened. So far, we have measured serum 1,25-$(OH)_2$D only in a pilot sample of 12 of our adult Asians with serum 25-(OH)D of less than 12.5 nmol/litre. In one individual the level was at the extreme upper limit of normal and in another it was unequivocally subnormal; in the rest, the serum 1,25-$(OH)_2$D was in the middle of the normal range.

The Genesis of Bone Disease in Vitamin D Deficiency

It can reasonably be argued that the reactive hyperparathyroidism in these severely vitamin D depleted individuals would maintain the serum calcium at the expense of a reduced bone mass [76]. There are, however, indications that secondary hyperparathyroidism may come into operation at a much earlier stage of vitamin D depletion. Among our surveyed Asians there were 62 other adults in whom the serum 25-(OH)D was between 12.5 and 25 nmol/litre. As a group, they showed a lowering of the serum calcium, the serum phosphate and the urinary calcium/creatinine ratio in a pattern virtually identical with that already demonstrated (*see* Figs 8 and 9) in the more severely vitamin D depleted group. It is evident that a reduction of serum 25-(OH)D becomes functionally significant—presumably by causing a lowering of serum 1,25-$(OH)_2$D—at concentrations considerably higher than the 10 nmol/litre conventionally associated with the threat of developing osteomalacia. The effect of secondary hyperparathyroidism in boosting the levels of serum 1,25-$(OH)_2$D could thus be operating over a prolonged period of time as the serum 25-(OH)D level diminishes slowly.

The parallel effect of prolonged hyperparathyroidism in inducing new bone remodelling units [77] could pro-

duce a significant degree of osteoporosis which, in older individuals, might well be irreversible [78]. In this regard it is interesting to recall that Garner and Ball [79] produced histological evidence that osteomalacia in patients with intestinal malabsorption was preceded by the development of osteoporosis; and Nordin has insisted repeatedly that vitamin D depletion may contribute to the production of osteoporosis in the elderly.

But it is, of course, the development of osteomalacia that is regarded as the characteristic consequence of vitamin D deficiency, and it is implicit from the previous discussion that I believe this to depend on the establishment of a critical deficiency of 1,25-$(OH)_2D$. Such deficiency causes homeostatic failure with a progressive reduction of the serum calcium and increasingly severe secondary hyperparathyroidism; the increase in bone turnover produced by this hyperparathyroidism results in the progressive replacement of mineralised by unmineralised bone and so the development of florid osteomalacia.

This statement begs the question of why the newly formed bone matrix should not mineralise normally. Reasons for rejecting the belief that this is simply the passive consequence of a reduced plasma [Ca] x [P] product have been given elsewhere [80, 81] and there is also evidence that hypophosphataemia cannot be held responsible [82]. In the rat, a species often reviled unjustly by the clinical osteologist as being totally irrelevant to his problems, there is the most convincing evidence that defective mineralisation of bone in vitamin D deficiency is due to hypocalcaemia, or to the effects of hypocalceamia on the calcium content of the osteoblast [83]. In the vitamin D replete rat, the induction of hypocalcaemia by parathyroidectomy also results in defective osseous mineralisation [84] and in both states the abnormality can be reversed by correcting the hypocalcaemia.

At first sight, it appears impossible to rationalise these experimental observations with the clinical counterpart in man. In the osteomalacia of chronic vitamin D deficiency, effective secondary hyperparathyroidism can restore the serum calcium virtually to normal without repairing the osteomalacia; and we have seen in hypocalcaemic osteomalacia that treatment with vitamin D will initiate healing of the bone disease even when the serum calcium is still grossly subnormal [28]. But, in the former situation the critical deficiency of 1,25-$(OH)_2D$ persists because the supply of its precursor is exhausted, whereas the latter situation is associated with greatly increased concentrations of circulating 1,25-$(OH)_2D$.

It appears that the concentration of serum 1,25-$(OH)_2D$ is the more important factor determining the mineralisation of osteoid in human osteomalacia, possibly by a direct action on the osteoblast to increase its content of calcium. It is firmly established that there is a cytoplasmic receptor for 1,25-$(OH)_2D_3$ in bone cells [85, 86] and preliminary autoradiographic studies by Stumpf and DeLuca, following the injection of radioactive 1,25-$(OH)_2D_3$ of very high specific activity, indicate that the label is accumulated selectively by the osteoblasts and some osteocytes (H. F. DeLuca, personal communication). Collateral evidence for this postulated direct role of 1,25-$(OH)_2D_3$ in the process of mineralisation is provided by recently published observations on the effects of pharmacological doses of the sterol in X-linked hypophosphataemic osteomalacia and rickets [87, 88]. The extent of the mineralisation front induced in the osteomalacic bone by this treatment was a direct function of the concentration of serum 1,25-$(OH)_2D$ attained.

Defective Osseous Mineralisation in Hypoparathyroidism

If the arguments developed are valid, one should find defective mineralisation of bone in clinical hypoparathyroidism since, in the untreated patient, concentrations of both the serum calcium and serum 1,25-$(OH)_2D$ may be very low. Morphological studies have documented the occurrence of defective osseous mineralisation in the parathyroidectomised dog [89], and Jowsey [90] has demonstrated the same in a few patients with hypoparathyroidism. So the expectation is justified, although the phenomenon has received little attention and no systematic study.

We find that there may be no osseous fixation of tetracycline in the untreated patient, and that oral treatment with 1,25-$(OH)_2D_3$ will restore a normal mineralisation front (unpublished observations). It is also characteristic of the therapeutic response to 1,25-$(OH)_2D_3$ that a period of 2-8 weeks of continued treatment may be required to restore the serum calcium to normal [91, 92]. In a few patients, we have measured a cumulative bodily retention of 10-12 g of calcium before the serum calcium becomes normal; and similar observations have been made in the past during treatment with dihydrotachysterol [93]. That quantity of calcium is greater than is needed to correct any calcium deficiency in the soft tissues and it is compatible with the repair of a moderate skeletal deficit of calcium. One does not say 'repair of a minor degree of osteomalacia' because in the strictest sense the bony lesion in hypoparathyroidism is not osteomalacia, which requires an increased bone turnover for its progressive development and ultimate clinical expression.

There are, however, a few reported cases of hypoparathyroidism in which the occurrence of osteomalacia has been suspected or actually proved. It is probably significant that most such cases have been in growing children or adolescents [94]; and, in the adult described by Albright in 1956 [95], he made a diagnosis of 'recurrent Graves' disease'. Bodily growth and hyperthyroidism are two influences that increase bone turnover in the absence of parathyroid hormone. Thus, although the conditioned deficiency of 1,25-$(OH)_2D$ in hypoparathyroidism appears to cause the expected defect of osseous mineralisation, it seems probable that clinical osteomalacia will develop only when there is operating some additional factor that increases the turnover of bone.

Summary

Vitamin D appears to influence parathyroid function indirectly through its effects on calcium metabolism

rather than by a direct action of its metabolites on the parathyroid glands. In states of both secondary and primary hyperparathyroidism, the quantitative production of 1,25-$(OH)_2$D may be determined by the prevailing concentration of serum 25-(OH)D but there appears to be some constraint that limits the formation of 1,25-$(OH)_2$D when the provision of its precursor exceeds the physiological. From the absence of this constraint in 'type 2 vitamin D dependency' it is inferred that it may operate through 'self-inhibition' of the renal production of 1,25-$(OH)_2$D. It is shown that the level of serum 25-(OH)D may always exert some influence on the production of 1,25-$(OH)_2$D and that this effect is facilitated by hyperparathyroidism. In developing vitamin D deficiency the reactive secondary hyperparathyroidism may thus function as an adaptive mechanism that sustains the level of serum 1,25-$(OH)_2$D in the face of a diminishing serum 25-(OH)D . Failure of this adaptation and the development of a critical deficiency of 1,25-$(OH)_2$D is regarded as the direct cause of defective mineralisation of bone. This concept would explain the absence of osteomalacia in some patients with very low levels of serum 25-(OH)D and the occurrence of defective osseous mineralisation in hypoparathyroidism.

Acknowledgements

This lecture relies heavily on the work of many colleagues among whom I would mention particularly Doctors E. B. Mawer, G. A. Lumb, C. M. Taylor, M. Davies and W. P. Stevens. Collectively, we are grateful to the Medical Research Council for continued support.

References

1. Albright, F. and Reinfenstein, E. C. Jr. (1948) *The Parathyroid Glands and Metabolic Bone Disease.* London: Baillière, Tindall and Cox.
2. DeLuca, H. F. and Schnoes, H. K. (1976) *Annual review of Biochemistry,* **45,** 631.
3. Fraser, D. R. and Kodicek, E. (1973) *Nature, New Biology,* **241,** 163.
4. Garabedian, M., Holick, M. F., DeLuca, H. F. and Boyle, I. T. (1972) *Proceedings of the National Academy of Science,* (USA), **69,** 1673.
5. Rasmussen, H., DeLuca, H. F., Arnaud, C., Hawker, C. and von Stendigk, M. (1963) *Journal of Clinical Investigation,* **42,** 1940.
6. Rasmussen, H. and Bordier, P. (1974) *The Physiological and Cellular Basis of Metabolic Bone Disease.* Baltimore: Williams and Wilkins.
7. Fraser, D. R. (1980) *Physiological Reviews,* **60,** 551.
8. Castillo, L., Tanaka, Y. and DeLuca, H. F. (1975) *Endocrinology,* **97,** 995.
9. Hughes, M. R., Brumbaugh, P. F., Haussler, M. R., Wergedal, J. E. and Baylink, D. J. (1975) *Science,* **190,** 578.
10. Bilezikian, J. P., Canfield, R. E., Jacobs, T. P., Polay, J. S., D'Adamo, A. P., Eisman, J. A. and DeLuca, H. F. (1978) *New England Journal of Medicine,* **299,** 437.
11. Adams, N. D., Gray, R. W. and Lemann, J. Jr. (1979) *Journal of Clinical Endocrinology and Metabolism,* **48,** 1008.
12. Eisman, J. A., Wark, J. D., Prince, R. L. and Moseley, J. M. (1979) *Lancet,* **2,** 931.
13. Haussler, M. R., Hughes, M. R., McCain, T. A., Zerwehk, J. E., Brumbaugh, P. F., Jubiz, W. and Wasserman, R. H. (1977) *Calcified Tissue Research* (Suppl.), **22,** 1.
14. Kaplan, R. A., Haussler, M. R., Deftos, L. J., Bone, H. and Pak, C. Y. C. (1977) *Journal of Clinical Investigation,* **59,** 756.
15. Broadus, A. E., Horst, R. L., Lang, R., Littledike, E. T. and Rasmussen, H. (1980) *New England Journal of Medicine,* **302,** 421.
16. Stanbury, S. W. (1968) In *Nutrition in Renal Disease,* p. 118. (ed G. M. Berlyne) Edinburgh: Livingstone.
17. Stanbury, S. W. (1980) in *Vitamin D: Molecular Biology and Clinical Nutrition,* p. 251 (ed A. W. Norman) New York: Marcel Dekker.
18. Mawer, E. B., Taylor, C. M., Backhouse, J., Lumb, G. A. and Stanbury, S. W. (1973) *Lancet,* **1,** 626.
19. Balsan, S. M., Garabedian, R., Sorgniard, R., Holick, M. F. and DeLuca, H. F. (1975) *Pediatric Research,* **9,** 586.
20. Stamp, T. C. B., Perry, W., MacArthur, S. and Jenkins, M. V. (1979) in *Vitamin D: Basic Research and its Clinical Application,* p.1153 (ed A. W. Norman, K. Schaefer, D. v. Herrath, H.-G. Grigoleit, J. W. Coburn, H. F. DeLuca, E. B. Mawer and T. Suda) Berlin: de Gruyter.
21. Peacock, M., Heyburn, P. J., Aaron, J. E., Taylor, G. A., Brown, W. B. and Speed, R. (1979) Ibid., p. 1177.
22. Bordier, P., Rasmussen, H., Marie, P., Miravet, L., Gueris, J. and Ryckwaert, A. (1978) *Journal of Clinical Endocrinology and Metabolism,* **46,** 284.
23. Eastwood, J. B., de Wardener, H. E., Gray, R. W. and Lemann, J. L. Jr. (1979) *Lancet,* **1,** 1377.
24. Ornoy, A., Goodwin, D., Noff, D. and Edelstein, S. (1978) *Nature, London,* **276,** 517.
25. Miravet, L., Redel, J., Carre, C., Queillé, M. L. and Bordier, P. (1976) *Calcified Tissue Research,* **21,** 145.
26. Mawer, E. B. (1980) *Clinics in Endocrinology and Metabolism,* **9,** 63.
27. Papapoulos, S. E., Clemens, T. L., Fraher, L. J., Gleed, J. and O'Riordan, J. L. H. (1980) *Lancet,* **2,** 612.
28. Stanbury, S. W., Taylor, C. M., Lumb, G. A., Mawer, E. B., Berry, J., Hann, J. and Wallace, J. (1981) *Mineral and Electrolyte Metabolism,* **5,** 212.
29. Gallagher, J. A. and Lawson, D. E. M. (1980) *Calcified Tissue International,* **31,** 215.
30. Haussler, M. R., Hughes, M. R., Pike, J. W. and McCain, T. A. (1977) in *Vitamin D: Biochemical, Chemical and Clinical Aspects related to Calcium Metabolism,* p. 473. (ed A. W. Norman, K. Schaefer, J. W. Coburn, H. F. DeLuca, D. Fraser, H.-G. Grigoleit and D. v. Herrath.) Berlin: de Gruyter.
31. Bouillon, R., Muls, E. and De Moor, P. (1980) *Journal of Clinical Investigation and Metabolism,* **51,** 793.
32. Stanbury, S. W., Davies, M., Lumb, G. A. and Mawer, E. B. (1981) to be published.
33. Marx, S. J., Spiegel, A. M., Brown, E. M., Koehler, J. O., Gardner, D. G., Brennan, M. F. and Aurbach, G. D. (1978) *American Journal of Medicine,* **65,** 235.
34. Davies, M., Klimiuk, P. S., Adams, P. H., Lumb, G. A., Large, D. M. and Anderson, D. C. (1981) *British Medical Journal,* **282,** 1023.
35. Hughes, M. R. and Haussler, M. R. (1978) *Journal of Biological Chemistry,* **253,** 1065.
36. Wechsler, W. R., Ross, F. P., Mason, R. S., Posen, S. and Norman, A. W. (1980) *Archives of Biochemistry and Biophysics,* **201,** 95.
37. Lumb, G. A. and Stanbury, S. W. (1974) *American Journal of Medicine,* **56,** 833.
38. Reiss, E. and Canterbury, J. M. (1969) *New England Journal of Medicine,* **280,** 1381.
39. Potts, J. T. Jr., Murray, T. M., Peacock, M., Niall, H. D., Tregear, G. W., Keutmann, H. T., Powell, D. and Deftos, L. J. (1971) *American Journal of Medicine,* **50,** 639.
40. Bouillon, R. and De Moor, P. (1977) *Journal of Clinical Endocrinology and Metabolism,* **45,** 261.
41. Habener, J. (1978) *Journal of Clinical Investigation,* **62,** 436.
42. Brown, E. M., Gardner, D. G., Brennan, M. F., Marx, S. J., Spiegel, A. M., Attie, M. F., Downs, R. W. Jr., Doppman, J. L. and Aurbach, G. D. (1979) *American Journal of Medicine,* **66,** 923.
43. Lloyd, H. M. (1968) *Medicine (Baltimore),* **47,** 53.
44. Prader, V. A., Illig, R. and Heierli, E. (1961) *Helvetica Paediatrica Acta,* **16,** 452.
45. Scriver, C. R. (1976) *Pediatrics,* **45,** 361.
46. Stanbury, S. W. and Mawer, E. B. (1978) In *Vitamin D,* p. 354 (ed D. E. M. Lawson) London: Academic Press.
47. Fraser, D., Kooh, S. W., Kind, H. P., Holick, M. F., Tanaka, Y. and DeLuca, H. F. (1973) *New England Journal of Medicine,* **289,** 817.

48. Fraser, D., Kooh, S. W. and Scriver, C. R. (1978) In *Endocrinology of Calcium Metabolism,* p.6. (ed D. H. Copp and R. V. Talmage.) Amsterdam: Excerpta Medica.
49. Reade, T. M., Scriver, C. R., Glorieux, F. H., Nogroby, B., Kelvin, E., Puirier, R., Holick, M. F. and DeLuca, H. F. (1975) *Pediatric Research,* **9,** 593.
50. Brooks, M. H., Bell, N. H., Love, L., Stern, P. H., Orfei, E., Queener, S. F., Hamstra, A. J. and DeLuca, H. F. (1978) *New England Journal of Medicine,* **298,** 996.
51. Liberman, U. A., Samuel, R., Halabe, A., Kauli, R., Edelstein, S., Weisman, Y., Papapoulos, S. E., Clemens, T. L., Fraher, L. J. and O'Riordan, J. L. H. (1980) *Lancet,* **1,** 504.
52. Balsan, S., Garabedian, M., Lieberherr, M., Gueris, J. and Ulmann, A. (1979) In *Vitamin D: Basic Research and its Clinical Application,* p. 1143. (ed A. W. Norman, K. Schaefer, D. v. Herrath, H.-G. Grigoleit, J. W. Coburn, H. F. DeLuca, E. B. Mawer and T. Suda.) Berlin: de Gruyter.
53. Marx, S. J., Spiegel, A. M., Brown, E. M., Gardner, D. G., Downs, R. W. Jr., Attie, M., Hamstra, A. J. and DeLuca, H. F. (1978) *Journal of Clinical Endocrinology and Metabolism,* **47,** 1303.
54. Rosen, J. F., Fleischman, A. R., Finberg, L., Hamstra, A. and DeLuca, H. F. (1979) *Pediatrics,* **94,** 729.
55. Tsuchiya, Y., Matsuo, N., Cho, H., Kumagai, M., Yasaka, A., Suda, T., Orimo, H. and Shiraki, M. (1980) *Journal of Clinical Endocrinology and Metabolism,* **51,** 685.
56. Zerwehk, J. E., Glass, K., Jowsey, J. and Pak, C. Y. C. (1979) *Journal of Clinical Endocrinology and Metabolism,* **49,** 171.
57. Colston, K. W. and Feldman, D. (1979) *Journal of Clinical Endocrinology and Metabolism,* **49,** 789.
58. Larkins, R. G., MacAuley, S. J. and MacIntyre, I. (1974) *Nature, London,* **252,** 412.
59. Colston, K. W., Evans, I. M. A., Spelsberg, T. C. and MacIntyre, I. (1977) *Biochemical Journal,* **164,** 83.
60. Omdahl, J. L. and Hunsaker, L. A. (1978) *Biochemical and Biophysical Research Communication,* **81,** 1073.
61. Lund, Bj., Sørensen, O. H., Lund, Bi., Bishop, J. E. and Norman, A. W. (1980) *Journal of Clinical Endocrinology and Metabolism,* **51,** 606.
62. Hughes, M. R., Baylink, D. J., Jones, P. G. and Haussler, M. R. (1976) *Journal of Clinical Investigation,* **58,** 61.
63. Horst, R. L. and Littledike, E. T. (1979) In *Vitamin D: Basic Research and its Clinical Application,* p. 999. (ed A. W. Norman, K. Schaefer, D. V. Herrath, H.-G. Grigoleit, J. W. Coburn, H. F. DeLuca, E. B. Mawer and T. Suda.) Berlin: de Gruyter.
64. Papapoulos, S. E., Clemens, T. L., Fraher, L. J., Lewin, I. G. Sandler, L. M. and O'Riordan, J. L. H. (1979) *Lancet,* **1,** 627.
65. Lund, Bj., Clausen, E., Friedberg, M., Lund, Bi., Moszkowicz, M., Neilsen, S. P. and Sørensen, O. H. (1980) *Nephron,* **25,** 30.
66. Mawer, E. B., Backhouse, J., Lumb, G. A. and Stanbury, S. W. (1971) *Nature, New Biology,* **232,** 188.
67. Bell, N. H., Stern, P. H., Pantzer, E., Sinha, T. K. and DeLuca, H. F. (1979) *Journal of Clinical Investigation,* **64,** 218.
68. Stern, P. H., De Olazabal, J. and Bell, N. H. (1980) *Journal of Clinical Investigation,* **66,** 852.
69. Gray, R. W., Lemann, J. Jr. and Adams, N. D. (1979) Ibid., p. 545.
70. McCance, R. M. and Widdowson, E. M. (1943) *Journal of Physiology,* **102,** 42.
71. Malm, O. J. (1958) *Scandinavian Journal of Clinical and Laboratory Investigation,* **10,** Suppl.-36.
72. Robertson, W. G., Gallagher, J. C., Marshall, D. H., Peacock, M. and Nordin, B. E. C. (1974) *British Medical Journal,* **4,** 436.
73. Davie, M., Lawson, D. E. M. and Jung, R. T. (1978) *Lancet,* **1,** 820.
74. Stanbury, S. W. and Mawer, E. B. (1980) *Clinical Science,* **58,** 523.
75. Stephens, W. P., Klimiuk, P. S., Lumb, G. A., Mawer, E. B. and Stanbury, S. W. (1981) to be published.
76. Arnaud, S. B., Arnaud, C. D., Bordier, P. J., Goldsmith, R. S. and Flueck, J. A. (1975) In *Vitamin D and Problems related to Uremic Bone Disease,* p. 397. (ed A. W. Norman, K. Schaefer, H.-G. Grigoleit, D. v. Herrath and E. Ritz.) Berlin: de Gruyter.
77. Frost, H. M. (1963) *Bone Remodelling Dynamics,* Springfield, Illinois: C. C. Thomas.
78. Parfitt, A. M. (1976) *Metabolism,* **25,** 1033.
79. Garner, A. and Ball, J. (1966) *Journal of Pathology and Bacteriology,* **91,** 545.
80. Stanbury, S. W. (1962) *Swiss Medical Journal,* **92,** 883.
81. Stanbury, S. W. (1972) *Clinics in Endocrinology and Metabolism,* **1,** 239.
82. Bordier, P., Tun-Chot, S., Martin, J., Queillé, M. L. and Hioco, D. (1971) In *Phosphate et Métabolisme Phosphocalcique,* p. 79. Paris: L'Expansion Scientifique Française.
83. Baylink, D., Stauffer, M., Wergedal, J. and C. Rich, (1970) *Journal of Clinical Investigation,* **49,** 1122.
84. Wergedal, J., Stauffer, M., Baylink, D. and C. Rich (1973) *Journal of Clinical Investigation,* **52,** 1052.
85. Kream, B. E., Jose, M., Yamada, S. and DeLuca, H. F. (1977) *Science,* **197,** 1086.
86. Manolagas, S. C., Taylor, C. M. and Anderson, D. C. (1979) *Journal of Endocrinology,* **80,** 35.
87. Drezner, M. K., Lyles, K. W., Haussler, M. R. and Harrelson, J. M. (1980) *Journal of Clinical Investigation,* **66,** 1020.
88. Glorieux, F. H., Marie, P. J., Pettifer, J. M. and Delvin, E. E. (1980) *New England Journal of Medicine,* **303,** 1023.
89. Burkhart, J. M. and Jowsey, J. (1966) *Mayo Clinic Proceedings,* **41,** 663.
90. Jowsey, J. (1968) In *Parathyroid Hormone and Thyrocalcitonin (Calcitonin),* p. 137. (ed R. V. Talmage and L. E. Belanger.) Amsterdam: Excerpta Medica.
91. Kooh, S. W., Fraser, D., DeLuca, H. F., Holick, M. F., Belsey, R. E., Clark, M. B. and Murray, T. M. (1975) *New England Journal of Medicine,* **293,** 840.
92. Davies, M., Hill, L. F., Taylor, C. M. and Stanbury, S. W. (1977) *Lancet,* **1,** 54.
93. Emerson, K., Walsh, F. B. and Howard, J. E. (1941) *Annals of Internal Medicine,* **14,** 1256.
94. Drezner, M. K., Neelon, F. A., Jowsey, J. and Lebovitz, H. E. (1977) *Journal of Clinical Endocrinology and Metabolism,* **45,** 114.
95. Albright, F. (1956) *Journal of Clinical Endocrinology,* **16,** 419.