VITAMIN D METABOLISM AND ITS CLINICAL APPLICATION

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The recent advances in vitamin D research have included the unravelling of the major steps in its metabolism and a sounder understanding of the actions of vitamin D at a variety of target organs. We are now in a position where this new knowledge can be applied to understanding the basis of many clinical disorders and to improving the management of some of these. In addition, an appreciation of the metabolism and actions of vitamin D allows therapeutics with vitamin D-like compounds to be approached as an exercise in applied physiology in contrast to the more empirical approaches formerly used. The purpose of this review is to summarise those aspects of vitamin D metabolism which have clinical importance in understanding the pathogenesis and thus the treatment of disordered mineral metabolism.

METABOLISM OF VITAMIN D

There are two ways in which vitamin D₃ (cholecalciferol) is normally provided to man: it is produced in the skin as a result of ultraviolet irradiation of 7-dehydrocholesterol, and it is also derived from the intestinal absorption of the intact vitamin from the diet. Vitamin D₂ (ergocalciferol) is derived from plant sterols and used as a therapeutic agent or for the fortification of foods. Both vitamin D₃ and vitamin D₂ are inactive in many biological systems and must undergo a series of metabolic transformations before exerting effects at target tissues (for reviews see DeLuca 1976; Haussler and McCain 1977; Fraser 1980). The active metabolites of vitamin D can now be considered hormones in the sense that their production rates are controlled and they affect target tissues often at sites removed from their source of production. In common with many endocrine systems, disorders of vitamin D metabolism can arise because of changes in the secretion of the active hormones or due to changes in the sensitivity of target tissues.

Production of 25-hydroxyvitamin D (25-OHD). The first step in the metabolism of vitamin D₃ (or vitamin D₂) is its hydroxylation in the 25 position (Fig. 1) by 25-hydroxylase. This step occurs predominantly in the liver (Olson et al. 1976) and results in the formation of 25-hydroxyvitamin D (25-OHD). In plasma it is the major circulating metabolite of vitamin D, and is commonly measured to provide an index of vitamin D nutritional status. Some animal experiments have suggested that product inhibition of the hepatic 25-hydroxylase occurs so that when progressively larger doses of parent vitamin D are administered, proportionately less 25-OHD is produced (Fukushima et al. 1978; for review see Fraser 1980), but there are a number of reasons for believing that this potential site for regulation of vitamin D-like activity is not of major physiological importance in man. Thus, serum 25-OHD in man increases progressively as the intake of vitamin D is increased (Haddad and Stamp 1974) and very high levels are found in vitamin D toxicity. Moreover, the total amount of 25-OHD in the plasma underestimates the total formed since variable amounts accumulate in other tissues. Concentrations may be greater in skeletal muscle than in the serum (Mawer 1974) and vitamin D is preferentially stored in

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fatty tissues. Since 25-OHD will act only when presented to target tissue, its storage in such sites may tend to limit its biological effects by acting as a buffer to prevent abrupt changes in its plasma concentration. Access to storage sites may, therefore, be quantitatively more important in protecting against vitamin D toxicity than any regulation of the activity of the 25-hydroxylase enzyme.

It is a widely held view that 25-OHD, at physiological concentrations does not act directly on target organs but must be further metabolised before it can function. A possible exception is skeletal muscle where 25-OHD is concentrated and may exert direct metabolic effects (Mawer 1974; Birge and Haddad 1975). It has also been suggested that 25-OHD has actions on bone which are not reproduced by administration of other metabolites of vitamin D (Bordier et al. 1978) but such effects could be indirect.

Some of the 25-OHD formed is secreted into the bile, mainly in the form of glucuronides, a proportion of which may be reabsorbed (Arnaud et al. 1975; Mawer 1974, 1979). The significance and magnitude of this enterohepatic circulation is controversial but even if small, the continual losses of 25-OHD by defects in reabsorption from the gut could prove important in the aetiology of certain forms of vitamin D-refractory osteomalacia associated with chronic liver or bowel disease.

**Table 1.** Some characteristics of the major metabolites of vitamin D3 in man

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Plasma concentration (µg/l)</th>
<th>Turnover rate (t) (days)</th>
<th>Production rate (estimated) (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D3</td>
<td>4-30</td>
<td>1-2</td>
<td>--</td>
</tr>
<tr>
<td>25-OHD3</td>
<td>5-50</td>
<td>5-20</td>
<td>10</td>
</tr>
<tr>
<td>1,25(OH)2D3</td>
<td>0.02-0.04</td>
<td>1-3</td>
<td>0.2</td>
</tr>
<tr>
<td>24,25(OH)2D3</td>
<td>1.5</td>
<td>12-31</td>
<td>1</td>
</tr>
<tr>
<td>25,26(OH)2D3</td>
<td>0.2-1.2</td>
<td>3-6</td>
<td>1.5</td>
</tr>
<tr>
<td>1,24,25(OH)2D3</td>
<td>0.01</td>
<td>Unknown</td>
<td>Uncertain</td>
</tr>
</tbody>
</table>

Much experimental evidence suggests that the biological activity of 25-OHD, occurs predominantly by its further hydroxylation in the 1 or 24 or 26 position to form 1,25(OH)2D3 or 24,25(OH)2D3 or 25,26(OH)2D3. The majority of 25-OHD formed probably undergoes oxidation of the side chain in the liver by microsomal enzymes (Kumar, Harned and DeLuca 1976; Harned et al. 1976), disturbances in which may be important in the pathogenesis of drug-induced osteomalacia and hepatic bone disease.

The proportion of 25-OHD metabolised to dihydroxy metabolites is uncertain but indirect estimates suggest this is less than 30 per cent (Table 1). The metabolite which has undoubtedly aroused the greatest interest has been 1,25(OH)2D3 since experimental work has implicated its defective production in the pathogenesis of a variety of clinical disorders especially vitamin D-resistant states.

**Production of 1,25(OH)2D3.** In man, 1,25(OH)2D3 circulates at a plasma concentration approximately 1000-fold less than that of 25-OHD. The physiological production rate lies between 0.2 and 1.0 micrograms daily and it has a rapid half-life in the peripheral circulation (Table I). The major site of its production is the kidney (Fraser and Kodicek 1970), although 1α-hydroxylase activity has been demonstrated in the placenta, the decidua and bone (Weisman et al. 1979), which might be important sources of 1,25(OH)2D3 in pregnancy and in disorders of vitamin D metabolism. The amount of 1,25(OH)2D3 produced by the kidney is subject to close metabolic regulation by the renal 1α-hydroxylase. Thus, unlike 25-OHD, the production of 1,25(OH)2D3 is not normally increased by increasing concentrations of its precursor. Recently, further steps in the metabolism of 1,25(OH)2D3 have been elucidated (Kumar et al. 1976). It is not known whether or not the degradation of 1,25(OH)2D3 is subject to metabolic regulation.

1,25(OH)2D3 production is increased under conditions of deprivation of phosphate, calcium or vitamin D, or in the presence of high levels of parathyroid hormone. Various other hormones, including prolactin, oestrogens, insulin and growth hormone, may also stimulate 1,25(OH)2D3 production during growth, pregnancy or lactation (Fraser 1980; DeLuca 1976), but it is unclear whether these hormones act directly to stimulate 1α-hydroxylase or whether these actions are indirect by changing the concentrations of calcium, phosphate or hydrogen ions in the plasma or the kidneys.

In contrast, hyperphosphataemia, hypercalcaemia, lack of parathyroid hormone or the presence of sufficient 1,25(OH)2D3 result in diminished activity of renal 1α-hydroxylase and an increase in activity of the 24-hydroxylase. Glucocorticosteroids, the diphosphonate disodium etidronate, heparin and some metal ions such as cadmium, strontium and possibly lead also inhibit production of 1,25(OH)2D3, but as in the case of oestrogens and growth hormone these actions may be indirect.

The best-known target-organ effects of 1,25(OH)2D3 are on the intestine to stimulate absorption of both calcium and phosphate, and on bone to cause release of calcium and phosphate. Thus, in common with other hormonal systems, a negative feedback mechanism may exist whereby the effect of 1,25(OH)2D3 in raising plasma calcium and phosphate in turn inhibits its synthesis and secretion.

From this brief description of the properties of 1,25(OH)2D3 it can be seen why it is currently considered to be a hormone rather than a vitamin. In contrast, until recently the other dihydroxy metabolites of vitamin D,
24,25(OH)₂D₃ and 25,26(OH)₂D₃, have been thought to be the product of alternative pathways for 25-OHD₃ metabolism for inactivation of vitamin D-like activity. This view has arisen since, in many experimental systems, the biological activity of 24,25(OH)₂D₃ and 25,26(OH)₂D₃ is much less than that of 1,25(OH)₂D₃.

**Production of 24,25(OH)₂D₃.** The production of 24,25(OH)₂D₃ is favoured when repletion of calcium and phosphate inhibits the synthesis of 1,25(OH)₂D₃. Thus, under many experimental conditions, the capacity to produce 1,25(OH)₂D₃ or 24,25(OH)₂D₃ appears to be reciprocally related so that when conditions favour production of one, production of the other is depressed (Boyle, Gray and DeLuca 1971). The nature of this regulation and the finding that the concentration of 24,25(OH)₂D₃ in plasma is about 100 times higher than that of 1,25(OH)₂D₃ under physiological conditions suggests that 24,25(OH)₂D₃ may have some function which differs from 1,25(OH)₂D₃. Indeed, recent evidence suggests that this metabolite may have distinct functions of its own (reviewed later). Moreover, the production of 24,25(OH)₂D₃ is stimulated by 1,25(OH)₂D₃ (Taylor 1979) and some of the actions previously attributed to 1,25(OH)₂D₃ may be due to changes in the endogenous production and activity of 24,25(OH)₂D₃.

The early animal experiments suggested that, as in the case of 1,25(OH)₂D₃, the major site of production of 24,25(OH)₂D₃ is the kidney (Knutson and DeLuca 1974). However, other tissues such as cartilage, bone, placental and intestinal tissue can convert 25-OHD₃ to 24,25(OH)₂D₃ under certain experimental conditions (Garabedian et al. 1978; Kumar, Schnoes and DeLuca 1978; Weisman et al. 1979) and may, therefore, be among the extrarenal sites of potential importance. Most assays for 24,25(OH)₂D₃ detect low or absent levels in anephric patients, suggesting that the kidney is the main site for its synthesis (Taylor et al. 1978; Shepard et al. 1979). The sites for synthesis of vitamin D metabolites has some clinical relevance in unravelling the pathophysiology of disturbed mineral metabolism in chronic renal failure.

A vitamin D-binding α-globulin binds cholecalciferol and its metabolites (Haddad and Walgate 1976), though with different binding affinities. This affects the relative concentrations of the various metabolites free for interaction with target tissues. It is likely that differences in protein binding account in part for differences in metabolic clearance between the metabolites.

**Other metabolites.** The third dihydroxy metabolite found in plasma in man is 25,26(OH)₂D₃ (Table I) but it is not yet clear whether or not its production is under metabolic control. A 26-hydroxylase is present in renal tissue (Tanaka et al. 1978) but it is unlikely that it is synthesised solely by the kidney since plasma levels are normal in patients with chronic renal failure or even under anephric conditions when synthesis of 1,25(OH)₂D₃ is low or absent (Shepard et al. 1979).

A further metabolite may be produced in the kidney by 24-hydroxylation of 1,25(OH)₂D₃ to form 1,24,25(OH)₃D₃. Conversely 1,24,25(OH)₃D₃ may be produced by 1α-hydroxylation of 24,25(OH)₂D₃. The experimental conditions under which 1,24,25(OH)₃D₃ production can be demonstrated are often unphysiological, but it has recently been detected in very low concentrations in normal man (Clemens et al. 1982b). The renal production and activity of 1,24,25(OH)₃D₃ might be important under some conditions particularly when 1,25(OH)₂D₃ or 24,25(OH)₂D₃ are used therapeutically.

**Actions of Vitamin D**

Although the physiological effects of vitamin D on calcium metabolism have been long recognised, several mysteries remain about its precise mechanism of action (for review see Kanis, Guillem-Cumming and Russell 1981a). For example, the most conspicuous effect of vitamin D deficiency in man is the defective and delayed mineralisation of bone resulting in rickets or osteomalacia; but there is little convincing evidence that vitamin D or its metabolites have direct actions to promote mineralisation of bone. From a physiological point of view, the known actions of vitamin D can be thought of as providing calcium and phosphate (perhaps for mineralisation) by effects on gut, bone and kidney (Fig. 2) and possibly by modulating the secretion of other hormones such as parathyroid hormone and calcitonin.

**Intestine.** Vitamin D increases the intestinal absorption of both calcium and phosphate. Vitamin D at physiological concentrations is inert and must first be metabolised to 1,25(OH)₂D₃. Thus calcium absorption is low in
patients with low levels of 1,25(OH)_{2}D_{3}, for example in chronic renal failure and hypoparathyroidism, and can be restored by the administration of physiological doses of 1,25(OH)_{2}D_{3} (Brickman et al. 1976). During the past few years there has been much interest in defining the biochemical basis for these effects of vitamin D, especially on calcium transport (for reviews see Lawson 1978; Bikle, Morrissey and Zolock 1979). The action of 1,25(OH)_{2}D_{3} includes the enhanced synthesis of alkaline phosphatase and calcium-binding protein but the manner in which these proteins mediate intracellular and transcellular transport of calcium and phosphatase is unclear. Receptor proteins binding 1,25(OH)_{2}D_{3} have been found in the cytoplasm and nuclei of intestinal cells and the isolation of these receptor proteins has been used in the development of radioreceptor assays for 1,25(OH)_{2}D_{3}. It is important to note that a wide range of vitamin D-like compounds and metabolites may compete with 1,25(OH)_{2}D_{3} for binding at nuclear receptor sites, though much higher concentrations are required. These agents may also therefore stimulate calcium transport under certain circumstances. For example, the administration of vitamin D or 25-OHD_{3} in pharmacological doses increases calcium absorption in anephric patients in whom the ability to convert 25-OHD_{3} to 1,25(OH)_{2}D_{3} is lost.

More recently it has become evident that small doses (1 to 20 micrograms daily) of 24,25(OH)_{2}D_{3} can also augment calcium absorption and whole-body retention of calcium in man (Kanis et al. 1977b, 1978b; Llach, Brickman and Coburn 1980). This is perhaps surprising since in animals the intestinal receptors bind 24,25(OH)_{2}D_{3} much less effectively than 1,25(OH)_{2}D_{3}, and in animals the former has been shown to have little effect on the transport of calcium in the gut. In man, however, plasma 24,25(OH)_{2}D_{3} circulates at levels approximately 100-fold greater than 1,25(OH)_{2}D_{3} and doses as small as two micrograms daily double plasma levels of this metabolite. This is because 24,25(OH)_{2}D_{3} has a metabolic half-life measured in days to weeks compared to that of 1,25(OH)_{2}D_{3} of several hours (Table I; Gray et al. 1978; Kanis et al. 1981b). These high plasma levels and slow metabolic turnover of 24,25(OH)_{2}D_{3} in man may explain in part its apparently high potency.

Comparisons of potency between metabolites can also be influenced by differences in further metabolism of the agent. For example, the effects of 24,25(OH)_{2}D_{3} on intestinal absorption of calcium in vitamin D-deficient animals appears to depend in part on conversion by the kidney to 1,24,25(OH)_{3}D_{3} (Boyle et al. 1973). Therefore in many experimental studies, nephrectomy has been found to reduce the apparent potency of 24,25(OH)_{2}D_{3}. In man this may not be the case, since anephric patients may also show increased calcium retention when given 24,25(OH)_{2}D_{3} (Kanis et al. 1977b, 1978b). Moreover, the oral administration of 1,24,25(OH)_{3}D_{3} to man has little or no immediate effect on calcium absorption (Cundy et al. 1979).

**Bone formation and mineralisation.** Although vitamin D deficiency results in impaired skeletal growth and defective mineralisation, there is no unequivocal evidence that 1,25(OH)_{2}D_{3} or other metabolites of vitamin D have direct effects on bone or cartilage to cause skeletal growth or mineralisation. This is partly because it is extremely difficult to study mineralisation *in vitro*, whilst *in vivo* the administration of vitamin D or 1,25(OH)_{2}D_{3} produces elevations in plasma calcium and phosphate that may themselves be responsible for mineralisation.

There are several clinical disorders which indicate the lack of any simple relationship between 1,25(OH)_{2}D_{3} and skeletal mineralisation (Table II). Thus in chronic

<table>
<thead>
<tr>
<th>Condition</th>
<th>Plasma phosphate</th>
<th>Plasma 1,25(OH)<em>{2}D</em>{3}</th>
<th>Osteoid mineralisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D deficiency</td>
<td>Low</td>
<td>Low</td>
<td>Impaired</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td>High</td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>Renal failure (anephric)</td>
<td>Usually high</td>
<td>Absent</td>
<td>Normal (in a variable proportion of patients)</td>
</tr>
<tr>
<td>Vitamin D-resistant rickets</td>
<td>Low</td>
<td>Normal</td>
<td>Impaired</td>
</tr>
<tr>
<td>Vitamin D-dependent rickets, Type I</td>
<td>Low</td>
<td>Low</td>
<td>Impaired</td>
</tr>
<tr>
<td>Vitamin D-dependent rickets, Type II</td>
<td>Low</td>
<td>Normal</td>
<td>Impaired</td>
</tr>
<tr>
<td>Phosphate deprivation</td>
<td>Low</td>
<td>High</td>
<td>Impaired</td>
</tr>
</tbody>
</table>

renal failure concentrations of 1,25(OH)_{2}D_{3} are invariably low and may be absent in anephric patients, but osteomalacia is a relatively infrequent occurrence (Kanis et al. 1977a) and mineralisation may be normal (Bordier et al. 1973). Furthermore, in several forms of vitamin D-resistant rickets due to defective tubular reabsorption of phosphate, skeletal mineralisation is impaired despite the presence of 1,25(OH)_{2}D_{3} and can be partially restored when plasma phosphate levels are raised by dietary phosphate. An inverse relationship between phosphate and the amount of osteoid is seen not only in vitamin D-resistant rickets but also in Paget's disease and in patients with chronic renal failure (Kanis et al. 1977a). Even in simple vitamin D deficiency it is possible that the low levels of plasma phosphate (due to secondary hyperparathyroidism) are primarily responsible for the mineralisation defect. There is some evidence that phosphate alone can restore mineralisation in vitamin D-deficient humans.
With regard to calcium concentrations, the situation is less clear but calcium deficiency rickets has been shown in man (Pettifor et al. 1979). The critical effect of calcium availability on mineralisation is shown in dietary calcium deficiency where osteomalacia fails to respond to 1,25(OH)₂D₃ until dietary calcium is supplied (Fig. 3; Cundy et al. 1982). Observations of this type suggest that bone mineralisation depends upon adequate supplies of both calcium and phosphate, and that 1,25(OH)₂D₃ acts by promoting the availability of both these ions.

Cartilage also appears to be a potential site of synthesis of 24,25(OH)₂D₃ (Garabedian et al. 1978). More recently it has been shown that normal differentiation of epiphyseal cartilage requires the presence of 24,25(OH)₂D₃ whereas 1,25(OH)₂D₃ alone is without effect (Ornay et al. 1979). It is important, however, to note that the processes which govern remodelling of the adult skeleton and those which operate to promote cartilage growth and mineralisation in the developing skeleton may be quite different. Thus the relevance of these skeletal actions of 24,25(OH)₂D₃ to the mature skeleton is uncertain.

Investigations of the effects of 24,25(OH)₂D₃ on mature bone have been largely confined to man. Balance studies in man, and more recently in dogs, have shown that 24,25(OH)₂D₃ acutely increases calcium retention and the accretion rate of calcium into bone (Kanis et al. 1977b, 1978b; Llach et al. 1980; Canterbury et al. 1980). In short-term studies plasma and urine calcium levels do not rise, indeed they commonly fall despite increases in the net intestinal absorption of calcium. This contrasts with 1,25(OH)₂D₃ or its synthetic analogue 1α-OHD₃ which do not lead to such marked calcium retention because the increment of calcium absorbed is largely excreted in the urine.

Longer term studies in patients with osteomalacia due to vitamin D deficiency have shown that the administration of 25-OHD₃ appears to restore defective mineralisation more completely than 1α-OHD₃ or 1,25(OH)₂D₃ alone (Bordier et al. 1978; Rasmussen and Bordier 1980). The more complete action of 25-OHD₃ is mimicked by the concomitant administration of 1,25(OH)₂D₃ and 24,25(OH)₂D₃. These findings suggest that the presence of both 1,25(OH)₂D₃ and 24,25(OH)₂D₃ may be necessary for remineralisation of osteoid.

These observations conflict with several studies showing that 1,25(OH)₂D₃ can reverse the biochemical and radiographic features of osteomalacia without raising plasma levels of 25-OHD₃ or 24,25(OH)₂D₃ (Peacock et al. 1979). However, little information is available about the histological effects which are of critical importance in determining whether 1,25(OH)₂D₃ alone can satisfy all the requirements for normal bone mineralisation in adult man.

Long-term responses to 24,25(OH)₂D₃ alone have been reported in patients with osteoporosis and patients with renal osteodystrophy. In osteoporotic patients the acute increase in calcium retention is not sustained (Reeve et al. 1982). In renal bone disease the responses have been variable which is perhaps not surprising considering its heterogeneous nature, but osteomalacia is not healed (Kanis et al. 1980). Consistent findings include an increase in calcium retention, little change or a fall in plasma calcium levels, and a rise in plasma alkaline phosphatase level. Plasma hydroxyproline values do not change over a three-month period of treatment suggesting that bone formation may be increased independently of
bone resorption (Kanis et al. 1980). Support for this suggestion must await long-term studies of the histological responses in bone.

These various observations do not show an unequivocal action of 24,25(OH)2D3 on bone formation or mineralisation in adult bone. They do, however, suggest that this metabolite may have acute skeletal effects, either direct or indirect, which differ from those of 1,25(OH)2D3. The small doses needed to achieve such effects suggest that these actions may have physiological relevance.

**Bone resorption.** One of the most marked effects of 1,25(OH)2D3 in animal models is to increase bone resorption both in vitro and in vivo (Reynolds, Holick and DeLuca 1974; Raisz et al. 1972). This action is not necessarily physiological and there is still a question as to whether this is an important effect in man. There is some evidence that doses of 1,25(OH)2D3 at the upper limit of those customarily applied may have some effect on bone resorption as seen by increased excretion of hydroxyproline and loss of total body calcium (Russell et al. 1974; Marshall and Nordin 1977). The increased bone resorption seen in some patients with sarcoidosis is also associated with high plasma levels of 1,25(OH)2D3. This may mean that doses of 1,25(OH)2D3 only slightly higher than physiological can induce bone resorption.

There is no evidence in man that 24,25(OH)2D3 augments bone resorption (Kanis et al. 1978b, 1980) and in patients with chronic renal failure levels of alkaline phosphatase may rise, whereas those of plasma hydroxyproline remain static.

**Muscle.** One of the striking clinical features of vitamin D deficiency is muscle weakness, mainly in the form of a proximal myopathy. In man, 1,25(OH)2D3 restores muscle strength rapidly when given, for example, to patients with osteomalacia or renal osteodystrophy. Despite these impressive clinical responses, there is still a lack of experimental data indicating a direct effect of vitamin D on muscle, although there is some evidence that sarcoplasmic calcium ATPase activity and troponin C concentrations may be partially dependent on vitamin D (Pleasure et al. 1979; Pointon, Francis and Smith 1979).

25-OHD3 is found in greater concentrations in muscle than in other tissues, and sites binding 25-OHD3 in preference to 1,25(OH)2D3 have been identified (Birge and Haddad 1975). Questions, however, remain concerning the dependence of muscle function on vitamin D itself rather than on availability of calcium and phosphate.

**Parathyroid glands.** Receptors for 1,25(OH)2D3 have been identified in parathyroid tissue (Henry and Norman 1975) and the long-term administration of 1,25(OH)2D3 or 1α-OHD3 to man commonly results in the suppression of plasma parathyroid hormone concentrations. This is particularly striking in patients with chronic renal failure with secondary hyperparathyroidism (Kanis et al. 1979b).

It is likely, however, that this is usually due to the suppression of parathyroid hormone secretion by raising plasma calcium concentrations rather than a direct effect of 1,25(OH)2D3 (see Fig. 3).

Several investigations have shown that in experimental animals and in man 24,25(OH)2D3 can suppress the secretion of parathyroid hormone (Canterbury et al. 1978, 1979; Miravet et al. 1981) but this effect has not been consistently reproduced by others (Heynen et al. 1981). The doses used have generally been large and may represent a pharmacological rather than physiological effect.

**Kidney.** The question of whether metabolites of vitamin D have direct effects on the renal handling of calcium and phosphate is difficult to study since intact animals have to be used and indirect effects are not easy to eliminate (for review see Bonjour and Fleisch 1977; Sutton and Dirks 1978; Kanis et al. 1981a). Thus renal tubular reabsorption of phosphate may fall in patients with hypoparathyroidism given 1,25(OH)2D3 but these effects may be indirect due to changes in plasma calcium levels. Vitamin D and its metabolites increase renal tubular reabsorption of calcium in man but once again this is unlikely to be a direct effect.

**Other target organs.** Classical steroid receptors for vitamin D metabolites have been identified in a number of other tissues including breast, pancreas, pituitary and placenta, and a vitamin D-dependent calcium-binding protein identified in several others. This suggests that vitamin D has trophic actions on many tissues the nature of which is still unclear. It is likely that such functions would not be related to conservation of calcium and phosphate.

**Integrated actions**

The multiple actions of the various vitamin D metabolites are slowly being clarified but much work clearly remains to be done concerning these actions in man. It should also be noted that even when biological effects of a vitamin D metabolite are demonstrated in man this need not necessarily represent the physiological action of the agent under normal conditions.

Although the actions of 1,25(OH)2D3 have been extensively studied, its production rate under physiological conditions in human adults is low except in pregnancy, and 24,25(OH)2D3 is the major circulating dihydroxy metabolite produced "electively" under physiological conditions. With the exception of the effects of 1,25(OH)2D3 to enhance intestinal transport of calcium and phosphate, there is not yet convincing evidence that effects of 1,25(OH)2D3 on bone, kidney or parathyroid gland represent actions of physiological relevance.

Nevertheless, from a consideration of the known and possible target-organ effects of 1,25(OH)2D3 and 24,25(OH)2D3 it is reasonable to speculate that 1,25(OH)2D3 may be involved in the maintenance of plasma calcium and phosphate since hypocalcaemia and...
hypophosphataemia stimulate its production, and its actions raise plasma levels of these ions.

The combined target-organ effects of 24,25(OH)₂D₃ in man suggest that it has an anabolic effect on the skeleton rather than direct actions of hypophosphataemia provide the presence of its receptor in bone. The observed increases in the plasma levels of 25(OH)D and 1,25(OH)₂D₃ suggest that these are not caused by increased intestinal absorption of vitamin D. These increases are also observed in summer when the plasma levels of vitamin D metabolites other than 25(OH)D are lower.

relevance to extracellular calcium and phosphorus homoeostasis. These actions, together with its slow turnover time in plasma, are properties ideal for a long-acting stimulator of bone formation. The regulation of renal 24-hydroxylase by 1,25(OH)₂D₃ suggests that one of the signals for 24,25(OH)₂D₃ synthesis could be the presence of amounts of 1,25(OH)₂D₃. The effects of 24,25(OH)₂D₃ might also depend in part on the actions of 1,25(OH)₂D₃ to provide calcium and phosphate.

The view that metabolites other than 1,25(OH)₂D₃ have physiological importance in man is strengthened by physiological studies of seasonal variations in mineral homoeostasis (Clayton et al. 1982). In northern Europe there are marked seasonal variations in serum and urinary concentrations of calcium and phosphorus, with increases in the summer months. Increases in the mineral content of metacarpal bone and in calcium balance are also observed in summer, which are associated with comparable changes in 25-OHD₃ and 24,25(OH)₂D₃ levels. In contrast, 1,25(OH)₂D₃ levels have generally been reported to decrease or remain unchanged in summer (Fig. 4), suggesting that if these changes in mineral metabolism are vitamin D-dependent, they must be attributable to changes in levels of vitamin D metabolites other than 1,25(OH)₂D₃.

Fig. 4
Serum concentrations (mean ± SEM) of vitamin D metabolites grouped by trimester in seven normal subjects. Also shown is metacarpal bone mineral content (B.M.C.) in 74 postmenopausal women. Note the marked increase of 25-OHD and 24,25(OH)₂D and bone mineral content during the summer months and in contrast the fall in mean serum concentration of 1,25(OH)₂D₃.

DISORDERS OF VITAMIN D METABOLISM
Clinical disorders can arise as a result of disturbances in any of the steps leading to the synthesis or action of vitamin D metabolites, particularly 1,25(OH)₂D₃. The mechanisms for the pathogenesis of these endocrine abnormalities follows the pattern common to many other endocrine systems (Table III).

Increased hormone levels
Increased production of 1,25(OH)₂D₃ may be found in several physiological and pathological states and is almost invariably associated with increased intestinal absorption of calcium (Table IV). The hormones known to increase production of 1,25(OH)₂D₃ include oestrogens, prolactin, growth hormone, and placental lactogen, but it is likely that these actions are secondary to other metabolic consequences of the hormone, for example periosteal bone growth in the case of growth hormone. This might explain the variable levels of 1,25(OH)₂D₃ reported in acromegaly and in prolactinoma.

Parathyroid hormone also stimulates the 1α-hydroxylase enzyme and increased levels of 1,25(OH)₂D₃ are also found in some, though not all, patients with primary hyperparathyroidism. Patients with normal levels of 1,25(OH)₂D₃ have lower urinary excretion rates for
VITAMIN D METABOLISM AND ITS CLINICAL APPLICATION

Table III. Mechanisms for disturbed metabolism of vitamin D

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Example of clinical disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Increased hormone levels</td>
<td></td>
</tr>
<tr>
<td>(i) Exogenous administration</td>
<td>Vitamin D toxicity</td>
</tr>
<tr>
<td>(ii) Increased plasma protein binding</td>
<td>Early pregnancy and oestrogens</td>
</tr>
<tr>
<td>(iii) Physiologically “appropriate” stimulus</td>
<td>Hyperparathyroidism</td>
</tr>
<tr>
<td>(iv) Ectopic hormone production</td>
<td>Sarcoidosis</td>
</tr>
<tr>
<td>B. Decreased hormone levels</td>
<td></td>
</tr>
<tr>
<td>(i) Increased catabolism</td>
<td>?Anticonvulsant osteomalacia</td>
</tr>
<tr>
<td>(ii) Increased excretion</td>
<td>Nephrotic syndrome</td>
</tr>
<tr>
<td>(iii) Absence of enzyme</td>
<td>Vitamin D-dependent rickets</td>
</tr>
<tr>
<td>(iv) Suppression of enzyme activity</td>
<td>Hypoparathyroidism</td>
</tr>
<tr>
<td>(v) Destruction of site of synthesis</td>
<td>Renal failure</td>
</tr>
<tr>
<td>(vi) Inappropriate suppression of enzyme</td>
<td>Oncogenic rickets</td>
</tr>
<tr>
<td>C. Decreased target-cell response</td>
<td></td>
</tr>
<tr>
<td>(i) Destruction of target tissue</td>
<td>Coeliac disease</td>
</tr>
<tr>
<td>(ii) Receptor or cell resistance</td>
<td>Anticonvulsant osteomalacia</td>
</tr>
<tr>
<td></td>
<td>Vitamin D-dependent rickets Type II</td>
</tr>
</tbody>
</table>

Table IV. States associated with high levels of 1,25(OH)₂D₃

| Physiological                              |                                                                   |
|                                           |                                                                   |
| Pregnancy and lactation                   |                                                                   |
| Skeletal growth                           |                                                                   |
| Appropriate stimulus                      |                                                                   |
| Acromegaly                                 |                                                                   |
| Prolactinoma                              |                                                                   |
| Oral contraceptives (*increased protein binding) |                                   |
| Primary hyperparathyroidism              |                                                                   |
| Hypocalcaemia (e.g. mithramycin, diphosphonates) |                                   |
| Phosphate depletion                        |                                                                   |
| Uncertain stimulus                        |                                                                   |
| Sarcoaidosis                               |                                                                   |
| Idiopathic hypercalciuria                 |                                                                   |
| Renal transplantation                      |                                                                   |
| Treatment of osteomalacia with vitamin D   |                                                                   |
| Glucocorticosteroids (acute only)          |                                                                   |
| Target organ insensitivity                 |                                                                   |
| Vitamin D-dependent rickets Type II       |                                                                   |
| Administration of 1,25(OH)₂D₃ or 1α-OHD₃  |                                                                   |

calcium. Since decreasing 1,25(OH)₂D₃ levels in patients taking phosphate supplements also decreases plasma and urine calcium and plasma alkaline phosphatase levels, it is probable that some of the manifestations of hyperparathyroidism may be due to effects of 1,25(OH)₂D₃.

Disturbances in 1α-hydroxylation have also been reported in idiopathic hypercalciuria (Caldas, Gray and Lemann 1978). A common finding in affected patients is a low plasma phosphate level due to decreased renal tubular reabsorption of phosphate. It is thought that elevated levels of 1,25(OH)₂D₃ found in some patients may be due to the hypophosphataemic stimulation of 1α-hydroxylase. As in primary hyperparathyroidism, administration of phosphate supplements may reduce urinary calcium excretion and lower the levels of 1,25(OH)₂D₃.

Increased production of 1,25(OH)₂D₃ occurs in some patients with sarcoidosis and probably accounts for the increased plasma calcium, intestinal calcium absorption and bone resorption noted in these patients (Pappouls et al. 1979). There is a marked seasonal variation in the incidence of hypercalcaemia due to sarcoidosis with the greatest incidence in summer. This observation and the finding of high 1,25(OH)₂D₃ levels after administration of vitamin D suggests that there is a failure of regulation of the 1α-hydroxylase enzyme. The mechanisms operating to increase the 1α-hydroxylase are unknown but it is likely that 1,25(OH)₂D₃ is synthesised by the sarcoid tissue since hypercalcaemia and high levels of 1,25(OH)₂D₃ have been noted in an anephric patient with sarcoidosis (Barbour et al. 1981).

With the exception of increased vitamin D sensitivity in sarcoidosis and toxicity from 1,25(OH)₂D₃ or 1α-OHD (converted in the body to 1,25(OH)₂D₃), it appears that vitamin D toxicity is not due to high circulating levels of 1,25(OH)₂D₃. Limited information from assays suggests that plasma 1,25(OH)₂D₃ levels are normal. Minor disturbances in 1α-hydroxylation may be present since low rather than normal levels might have been predicted under such circumstances. The hypercalcaemia of vitamin D and 25-OHD₃ overdose presumably reflects the pharmacological actions of high concentrations of these agents on gut, bone and kidney.

Deficiency of precursor metabolites of 1,25(OH)₂D₃
Clinical disorders can arise as a result of disturbances in any of the steps leading to the synthesis of 1,25-dihydroxycholecalciferol.

Simple deficiency of vitamin D. This arises from either inadequate dietary intake of vitamin D₃ or from inadequate exposure to ultraviolet light, which converts 7-dehydrocholesterol to endogenous vitamin D₃ in the skin. The natural diet contains small amounts of vitamin D₃ found mainly in dairy products. The diet may be supplemented with vitamin D₂ (ergocalciferol: an irradiation product from plant sterols) which is used to “fortify” foodstuffs such as margarine. The relative contribution of diet and sunlight to vitamin D status is
controversial, but the large seasonal fluctuations in 25-OHD levels in northern Europe suggest that solar irradiation provides the major source of vitamin D. Nevertheless, dietary deficiency coupled with inadequate exposure to sunlight must still be a critical factor in the pathophysiology of nutritional osteomalacia.

In Britain, nutritional osteomalacia is most commonly observed in the elderly, the institutionalised and in late pregnancy and adolescence, particularly in the Asian population. Dietary surveys of the elderly indicate a low intake of vitamin D (recommended intake 2.5 micrograms or 100 international units daily) but the recommended intake is probably too low if patients are not exposed to sunlight. Similar considerations apply to Asian immigrants since skin pigmentation interferes with the dermal production of vitamin D (Clemens et al. 1982a). It is notable, however, that rickets and osteomalacia are infrequent in the immigrant Negro population.

An additional factor in Asians may be the high phytate content of chapati flour which reduces the availability of dietary calcium for absorption. Osteomalacia refractory to treatment with 1,25(OH)2D3 has been described in an Asian with a calcium-deficient diet (Cundy et al. 1982). The osteomalacia responded to dietary calcium supplements suggesting that adequate dietary calcium may be critical for normal mineralisation.

Gastrointestinal and hepatic disorders. An increased prevalence of rickets and osteomalacia is seen in malabsorption syndromes, liver disease and after partial gastrectomy or jejun-ileal bypass (Compston et al. 1978; Compston and Thompson 1977; Melvin et al. 1970). Since vitamin D is fat-soluble it has been suggested that osteomalacia in steatorrhoea is due to inadequate absorption of vitamin D (Thompson, Lewis and Booth 1966). This is likely to be an oversimplification and the relative contributions of calcium malabsorption and disturbances in the enterohepatic circulation of vitamin D metabolites require assessment. In gluten-sensitive enteropathy (coeliac disease) and other intestinal disorders the disease destroys one of the target tissues for vitamin D, namely the intestinal villi. In such cases the gut is unable to respond to adequate amounts of 1,25(OH)2D3 even when given parenterally (Kanis, Russell and Smith 1977c).

It has been rather difficult to demonstrate unequivocally that disturbances in the hepatic conversion of vitamin D to 25-OHD contribute significantly to clinical disorders. Osteomalacia is described in chronic liver disease but bone biopsies suggest that osteoporosis is more frequently found. The administration of anticonvulsants which induce liver microsomal enzymes is associated with osteomalacia (Dent et al. 1970). Although this association with liver disease or anticonvulsant drugs may be due to disturbances in the formation of 25-OHD3 (Hahn et al. 1972; Silver 1977), alternative explanations are also possible. Thus, in hepatic osteodystrophy, intestinal malabsorption of vitamin D coupled with diminished exposure to sunlight often occurs. Plasma phosphate level is characteristically low in liver disease due to decreased tubular reabsorption of phosphate and it is possible that this contributes to osteomalacia. Both the significance and magnitude of the enterohepatic circulation of 25-OHD3 are controversial but disturbances in reabsorption of 25-OHD3 may contribute to low plasma levels in hepatic as well as in gastrointestinal disorders, particularly in patients taking cholestyramine (Compston and Horton 1978). In patients taking anticonvulsants, dietary deficiency of vitamin D and decreased ultraviolet irradiation (a feature of institutionalisation) may also be factors. There is experimental evidence that anticonvulsants interfere with the peripheral action of vitamin D metabolites on gut or bone and these actions may be of more importance for inducing bone disease than impairment of 25-hydroxylation (Jenkins, Harris and Wills 1974; Jubiz et al. 1977).

25-OHD and renal disease. Low plasma levels of 25-OHD3 have been reported in patients with chronic renal disease, especially in patients not yet on dialysis treatment. There is, however, no convincing evidence for defective formation of 25-OHD3, and low levels presumably reflect deficiency of vitamin D3 from inadequate sunlight or dietary deprivation. However, in patients with nephrotic syndrome, low plasma levels are associated with increased urinary losses of protein-bound 25-OHD3 which may give rise to bone disease (Malluche, Goldstein and Massry 1979).

Deficient production of 1,25(OH)2D3
The major interest in the use of 1,25(OH)2D3 and its synthetic analogues arises in those disorders where disturbances in the formation or action of 1,25(OH)2D3 occur despite adequate production and blood levels of 25-OHD3 (Table V). The degree of reduction of plasma

<table>
<thead>
<tr>
<th>Major clinical significance</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common</td>
<td></td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>Vitamin D-dependent rickets</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td>Type 1</td>
</tr>
<tr>
<td>Pseudohypoparathyroidism</td>
<td>Oncogenic osteomalacia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Uncertain clinical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs: corticosteroids, cadmium, heparin</td>
</tr>
<tr>
<td>Vitamin D-resistant rickets</td>
</tr>
<tr>
<td>Renal tubular disorders</td>
</tr>
<tr>
<td>Renal tubular acidosis</td>
</tr>
<tr>
<td>Cystinosis</td>
</tr>
<tr>
<td>Postmenopausal osteoporosis</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
</tr>
</tbody>
</table>

Table V. Disorders associated with low levels of 1,25(OH)2D3, but normal levels of 25-OHD3.
1,25(OH)2D3 in these disorders varies as does the significance of the low 1,25(OH)2D3 to the clinical features.

**Vitamin D-dependent rickets.** This is an extremely rare inherited autosomal recessive condition, also known as pseudo-vitamin D deficient rickets. It is characterised by the failure to convert 25-OHD3 to 1,25(OH)2D3 which has been attributed to an inherited deficiency of the 1α-hydroxylase enzyme in the kidney (Fraser et al. 1973; Scriver et al. 1978). Assays for 1,25(OH)2D3 have recently shown that levels of 1,25(OH)2D3 are not always low in patients with this clinical syndrome (Brooks et al. 1978). In such cases target-organ responses to 1,25(OH)2D3 may be impaired (vitamin D-dependent rickets Type II). A variant of Type II associated with distinctive somatic features (baldness and dental dysgenesis) has recently been recognised (Rosen et al. 1979).

**Chronic renal failure.** The manifestations of disturbed mineral metabolism in chronic renal failure include retarded growth, myopathy, hypocalcaemia, hyperphosphataemia, ectopic calcification and bone disease. The bone disease of renal failure comprises combinations of defective mineralisation of bone (osteomalacia and rickets), secondary hyperparathyroidism (osteitis fibrosa), osteosclerosis, periostal new bone formation and osteoporosis. Its aetiology is complex and the relative importance of disturbed metabolism of phosphate, vitamin D, parathyroid hormone, aluminium and other factors has been extensively reviewed (Coburn et al. 1977; Haussler and McCain 1977; Kanis 1978; Kanis et al. 1979a). It is probable that deficiency of 1,25(OH)2D3 accounts for the calcium malabsorption of chronic uraemia and, at least in part, for hyperparathyroidism, hypocalcaemia and osteomalacia.

Although destruction of, or damage to, renal tissue seems to be the most likely explanation for reduced 1,25(OH)2D3 levels, other explanations are possible. Thus, the elevated plasma phosphate level found in many of these patients may also contribute, particularly when reductions in glomerular filtration rate are modest and other endocrine functions of the kidney are preserved. It is possible that uraemic toxins, presently unidentified, may also be responsible and further work in this area is urgently needed. The relationship between the reduced 1,25(OH)2D3 and skeletal disease is far from simple, since not all patients with chronic renal failure exhibit skeletal abnormalities and other factors contribute to renal bone disease. Furthermore, anephric patients in whom 1,25(OH)2D3 levels are undetectable, do not have more marked bone disease than dialysis-treated patients with kidneys.

Renal osteodystrophy is very common though it may not give rise to clinical problems. Patients most at risk include children before the onset of skeletal maturity, and adults with interstitial forms of nephropathy, for example, analgesic nephropathy or chronic pyelonephritis. The advent of successful long-term dialysis treatment has also resulted in an increasing number of patients with clinically significant disturbances of mineral metabolism, though in some cases skeletal disease may be due to factors relating to the dialysis rather than to the uraemic state. Chronic renal failure was the first clinical disorder to be studied using the 1α-hydroxylated derivatives of vitamin D (Brickman, Coburn and Norman 1972; Henderson et al. 1974) and such patients comprise the largest group who have received the compounds.

**Hypoparathyroidism.** The clinical efficacy of 1,25(OH)2D3 in rapidly restoring plasma calcium in hypoparathyroid patients was well established before hypoparathyroidism was formally demonstrated to be a 1,25(OH)2D3 deficiency state (Russell et al. 1974). Several groups have now shown clearly that 1,25(OH)2D3 concentrations are low in postoperative and idiopathic hypoparathyroid patients. Two possible explanations seem likely. Firstly, since parathyroid hormone stimulates 1,25(OH)2D3 production in experimental animals and birds, the failure to convert 25-OHD3 to 1,25(OH)2D3 in human hypoparathyroidism may be attributable to deficiency of parathyroid hormone. An alternative explanation is that the high plasma phosphate level due to enhanced renal tubular reabsorption of phosphate in this disorder suppresses the renal conversion. The distinction between these two possibilities has not been resolved experimentally in man, though plasma calcium and calcium absorption can be increased by dietary deprivation of phosphate.

**Pseudohypoparathyroidism.** This disorder is due to defective target-organ responses to parathyroid hormone, which is present in normal or elevated concentrations. Despite the presence of parathyroid hormone, and in some patients of elevated prolactin levels, the conversion of 25-OHD3 to 1,25(OH)2D3 is defective (Drezner et al. 1976). Possible explanations include the elevated plasma phosphate level found in such patients, or defective renal responses of the 1α-hydroxylase to stimulation by parathyroid hormone.

**Vitamin D-resistant rickets.** Several distinct types of rickets or osteomalacia are associated with renal tubular disorders, which share the common feature of a reduced tubular reabsorption of phosphate. These include familial and sporadic forms of hypophosphataemic rickets as well as a host of much rarer diseases such as oncogenic osteomalacia, Fanconi syndrome and renal tubular acidosis. In some of these disorders levels of 1,25(OH)2D3 may be low. This is probably not the case in patients with the X-linked form of vitamin D-resistant rickets (Hausler et al. 1977); it seems more likely that the features of this disorder can be attributed to a low plasma phosphate level rather than to 1,25(OH)2D3 deficiency. Since hypophosphataemia in experimental animals seems to stimulate 1,25(OH)2D3 synthesis by the kidney, it is perhaps surprising that 1,25(OH)2D3 levels are not increased in this disorder. However, the concentrations of phosphate in renal tubular fluid are likely to be normal.
in this disorder and it is possible that renal tubular cells may also possess normal concentrations of phosphate. Osteomalacia associated with hypophosphataemia and low plasma levels of \(1,25(\text{OH})_2\text{D}_3\) has been well documented in patients with a variety of mesenchymal tumours. These are often exceedingly small. The interest in their identification is that surgical excision may reverse these biochemical abnormalities and heal the osteomalacia.

**Osteoporosis.** Plasma values of \(1,25(\text{OH})_2\text{D}_3\) reported in osteoporosis are normal or slightly reduced below values found in control subjects (Riggs and Gallagher 1977). With such small differences observed, the matching of patients with control subjects becomes critical. Nonetheless, the hypothesis that slight reductions in \(1,25(\text{OH})_2\text{D}_3\) levels could account for the reduced ability of such patients to absorb dietary calcium is attractive. There is evidence that oestrogen therapy can restore \(1,25(\text{OH})_2\text{D}_3\) concentrations and it is postulated that it is oestrogen deficiency that leads to the reduced values in osteoporosis. An alternative view is that osteoporosis occurs independently of disturbed vitamin D metabolism. The net bone resorption tends to raise plasma calcium. This calcium repletion (with respect to the extracellular fluid) combined with suppression of parathyroid hormone secretion might account for decreased 1α-hydroxylation of 25-OHD₃. This latter alternative could also account for the low values of \(1,25(\text{OH})_2\text{D}_3\) noted in osteoporosis due to Cushing's disease, steroid therapy (Klein et al. 1977), in thyrotoxicosis and after long-term treatment with heparin.

**Target-organ resistance.** Vitamin D-deficiency states may also arise when the target-organ responses to \(1,25(\text{OH})_2\text{D}_3\) are impaired. Impaired action of \(1,25(\text{OH})_2\text{D}_3\) probably occurs in untreated coeliac disease where intestinal absorption of calcium is not increased by relatively large doses of \(1,25(\text{OH})_2\text{D}_3\) even when given intravenously (Kanis et al. 1977c). The osteomalacia of anticonvulsant treatment probably reflects a drug-induced resistance of target organs (Jenkins et al. 1974) as well as defective metabolism of 25-OHD₃. Some patients with the clinical syndrome of vitamin D-dependent rickets have normal levels of \(1,25(\text{OH})_2\text{D}_3\) (VDDR Type II) and impaired mineralisation may therefore reflect target-organ resistance.

**Abnormalities of 24,25(OH)₂D₃ metabolism.** True deficiency states of 24,25(OH)₂D₃ with unique metabolic consequences have not been described with one possible exception (Liberman et al. 1980) although low levels occur in vitamin D-deficiency, chronic renal failure and possibly in osteoporosis (Weisman et al. 1978). Though 24,25(OH)₂D₃ levels are low in chronic renal failure and bone disease is common there may not be a causal relationship between these two findings. Such arguments have been previously used to argue a causal relationship between 1,25(OH)₂D₃ deficiency and renal osteodystrophy. If, as is thought, the kidney is the major site of synthesis of 24,25(OH)₂D₃ in adult man, the finding that anephric patients do not have a higher incidence of bone disease than patients with kidneys, and that mineralisation rates may be normal, would suggest that like \(1,25(\text{OH})_2\text{D}_3\) the presence of 24,25(OH)₂D₃ is not essential for the maintenance of skeletal integrity.

**THERAPEUTIC USE OF VITAMIN D-LIKE COMPOUNDS**

**Synthetic analogues of 1,25(OH)₂D₃**

The structural requirements for optimal vitamin D activity have been the subject of much research which has emphasised the importance of a hydroxyl group at carbon 1 of the A ring (Norman et al. 1976; see Fig. 5). Interest has focused on synthetic analogues of 1,25(OH)₂D₃ because of their clinical potential and because they were initially more readily synthesised than 1,25(OH)₂D₃. Two commercially available synthetic steroids, dihydrotachysterol (DHT) and 1α-hydroxycholecalciferol (1α-OHD₃) are biologically active without the necessity for 1α-hydroxylation by the kidney. 1α-OHD₃ is converted in the body to 1α,25(OH)₂D₃ (Holick et al. 1975; Fukushima et al. 1978). In the case of DHT the hydroxyl group responsible for biological activity is in the position of carbon atom 3 (Fig. 5), but the A ring is

![Fig 5](image_url)  
Structural formulae of vitamin D₃ and some related compounds.
rotated to give a pseudo 1α-hydroxyl group at the steric equivalent of carbon atom 1 (Norman et al. 1976).

DHT, like 1α-OHD3, also undergoes hepatic hydroxylation (Bhattacharyya and DeLuca 1973) and the 25-OHDHT so formed is the major circulating form of DHT (Hallick and DeLuca 1972). 25-OHDHT is at least twice as potent as DHT on a molar basis and the 25-hydroxyl and the pseudo 1α-hydroxyl groups give it a stereochemical configuration similar to 1,25(OH)2D3.

A host of other analogues and metabolites have been synthesised. The metabolites and analogues of vitamin D which are currently undergoing clinical investigation as potential therapeutic agents include 25,26(OH)2D3, 24,25(OH)2D3, 1,24(OH)2D3 and 25-OH-5,6-trans-D3. The latter compound has a steric configuration similar to 1,25(OH)2D3 and is biologically active in patients with chronic renal failure and hypoparathyroidism. However, as in the case of DHT much larger doses (150 to 450 micrograms daily) are required than with the use of 1α-OHD3 or 1,25(OH)2D3.

Are there advantages in 1,25(OH)2D3? A variety of vitamin D-like preparations are commercially available for use in clinical practice. These include vitamin D2, 25-OHD3 (not in Britain), 1α-OHD3 (One Alpha), 1,25(OH)2D3 (Rocaltrol) and DHT (Tachyrol). Apart from differences in the dose needed (Table VI), there is little evidence that any of these compounds have any particular therapeutic action not also possessed by vitamin D3. Manifestation of vitamin D deficiency in man (muscle weakness, hypocalcaemia, defective skeletal mineralisation and growth, bone pain, intestinal malabsorption of calcium and phosphate and secondary hyperparathyroidism), may be reversed as completely by vitamin D itself as by these analogues and metabolites. Similarly in chronic renal failure, vitamin D or 25-OHD3 seem effective in the treatment of renal bone disease (Teitelbaum et al. 1976; Stanbury and Lumb 1962; Pendras 1969) even in anephric patients (Kanis et al. 1977c) where conversion to 1,25(OH)2D3 is presumably absent.

**Table VI. Influence of a 1α-hydroxyl group (pseudo 1α-OH in the case of DHT) on daily dose requirements in the treatment of vitamin D-resistant disorders**

<table>
<thead>
<tr>
<th></th>
<th>D3</th>
<th>DHT</th>
<th>1,25(OH)2D3, or 1α-OHD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention or treatment of vitamin D deficiency rickets (μg)*</td>
<td>2.5 25</td>
<td>up to 200</td>
<td>up to 1.5</td>
</tr>
<tr>
<td>Relative potency</td>
<td>100</td>
<td>10</td>
<td>500</td>
</tr>
<tr>
<td>Treatment of hypoparathyroidism and other vitamin D-resistant states (μg)*</td>
<td>750 5000</td>
<td>200 1000</td>
<td>0.5 2.0</td>
</tr>
<tr>
<td>Relative potency</td>
<td>100</td>
<td>450</td>
<td>200 0000</td>
</tr>
</tbody>
</table>

* 1 μg D3 or 1,25(OH)2D3 is equivalent to approximately 40 iu.

Any advantage that vitamin D-like compounds have over the use of vitamin D itself must therefore depend more on factors such as the ease of use, possible side-effects and cost rather than on the dose required or the therapeutic result. This conclusion is supported by observations that patients with renal bone disease who fail to respond to adequate doses of vitamin D also fail to respond to 1α-OHD3 or 1,25(OH)2D3 (Prior et al. 1979; Kanis et al. 1979b). With vitamin D, difficulties often arise in finding the appropriate dose to use in vitamin D-resistant states since requirements between patients may vary widely, and requirements in individuals change abruptly (Pendras 1969; Parfitt and Frame 1972; Avioli 1974). This is perhaps not surprising since in hypoparathyroidism, for example, the metabolic defect may be incomplete and, in individuals, the capacity for 1α-hydroxylation may be intermittently restored.

Dose requirements for 1,25(OH)2D3, 1α-OHD3 and DHT may also vary in patients with severe bone disease though the phenomenon has been best documented in the case of 1α-OHD3 (Kanis et al. 1979b).

A major advantage in the use of 1,25(OH)2D3 and its synthetic analogues over vitamin D lies in the ease in titrating dose requirements (Figs 6 and 7). First, there is
clearly a wide variation in the amount of vitamin D required to treat the various vitamin D resistant states whereas the range with these agents is less (Parfitt and Frame 1972; see Table VI). Thus 0.5 to 3.0 micrograms of 1,25(OH)\(_2\)D\(_3\) daily (a six fold dose range) is sufficient with few exceptions to treat all vitamin D-related disorders, whereas with vitamin D a dose range of approximately 2000 (2.5 micrograms to 5.0 milligrams) is required (Table VI). Moreover, vitamin D\(_2\) preparations are only available in Britain as capsules containing 12.5 micrograms or 1.25 milligrams (500 or 50000 international units) which increases the difficulties in providing acceptable and safe dose regimes. A second advantage is that the rate of onset of action of 1,25(OH)\(_2\)D\(_3\), 1α-OHD\(_3\) and DHT is significantly more rapid than that of 25-OHD\(_3\) and the parent vitamins. Maximal effects are seen within one to three weeks (Fig. 7) compared with one to three months (Fig. 6) using vitamin D (Parfitt and Frame 1972; Parfitt 1978). With all vitamin D-like compounds there is a narrow range between requirements (the dose required to maintain a therapeutic response) and tolerance (the dose which just fails to give unacceptable hypercalcemia), and accidental hypercalcemia occurs commonly (Pendras 1969; Coburn et al. 1977; Kanis and Russell 1977). In the case of 1,25(OH)\(_2\)D\(_3\) and its synthetic analogues (DHT and 1α-OHD\(_3\)) the onset of hypercal-

Fig. 7

Long-term treatment of renal bone disease, a combination of osteomalacia (OM) and osteitis fibrosa (OF), with 1α-hydroxyvitamin D\(_3\), in a dialysis-treated patient. Healing of bone disease occurred within 15 months. Episodes of hypercalcemia occurred abruptly and the dose of 1α-OHD\(_3\) tolerated decreased progressively once plasma alkaline phosphatase had fallen to normal. A decrease in plasma immunoreactive parathyroid hormone (iPTH) also occurred. Remission from bone disease was maintained on one microgram of 1α-OHD\(_3\), thrice weekly.

Fig. 8

Rate of reversal of biological effects after stopping vitamin D\(_2\) and the 1α-hydroxylated derivatives of vitamin D\(_3\). The fall of plasma or urine calcium (observed minus asymptotic value) is shown on a logarithmic scale and expressed as a percentage of the initial value (from Kanis and Russell 1977).
not only in the management of vitamin D toxicity, but in allowing more rapid and precise adjustment of dose against requirements (Parfitt 1978; Sagar, Estrada and Kaye 1972; Heyburn and Peacock 1977; Kanis et al. 1977c).

**Treatment with 1,25(OH)₂D₃ and its analogues**

The clinical evaluation of 1,25(OH)₂D₃ was hampered initially by the very limited supplies of material available for clinical investigation. Indeed its more readily synthesised analogues, DHT and 1α-OHD₃, became available first.

**Simple vitamin D deficiency.** All these agents can replace vitamin D in simple vitamin D deficiency (Peacock et al. 1979; Jesserer and Swoboda 1959). However, there are advantages in administering vitamin D in simple vitamin D deficiency since the vitamin is subject to normal metabolic regulation, and the risks of toxicity are much decreased. In contrast, the 1α-hydroxylated derivatives bypass this regulation.

A much better case can be advanced for using 1,25(OH)₂D₃ in those clinical states which traditionally have required large doses of vitamin D₃ for treatment. The apparent "vitamin D resistance" in several of these (chronic renal failure, hypoparathyroidism, pseudohypoparathyroidism, vitamin D-dependent rickets) can now be understood because there is defective production of 1,25(OH)₂D₃ despite adequate levels of its immediate precursor, 25-OHDL. These are the four disorders in which the most impressive results have been seen with the use of 1α-hydroxylated derivatives of vitamin D.

**Chronic renal failure.** Impressive clinical effects of 1,25(OH)₂D₃ and related compounds are obtained in the majority of patients who have evidence of established renal bone disease (Kaye et al. 1970; Coburn et al. 1977; Madsen and Øgaard 1978; Kanis et al. 1979b). The commonest form of skeletal abnormality is attributable to secondary hyperparathyroidism, with its resultant radiographic and histological features. A most reliable biochemical indicator is a raised plasma alkaline phosphatase level. In about three-quarters of patients treatment induces a fall in plasma alkaline phosphatase, often into the normal range (for example see Fig. 7). Symptoms of bone pain and muscle weakness improve in about the same number, although changes in bone biopsies are less marked. The response of osteomalacia to treatment is more variable due to its variable aetiology in dialysis-treated patients.

The majority of patients who respond show a rise in plasma calcium values and many believe this to be an essential feature of a good response, since it is accompanied by a fall in parathyroid hormone concentrations. Those patients who start treatment with a high plasma calcium tolerate less drug, rapidly become hypercalcaemic and tend to respond less well than patients starting with a lower plasma calcium level.

Since 1,25(OH)₂D₃ levels are universally low in chronic renal failure, and not all patients have bone disease, it is too simplistic to view the response to 1,25(OH)₂D₃ as mere hormonal replacement therapy. The mode of action is probably mainly attributable to suppression of parathyroid hyperactivity, either by direct suppression of secretion of parathyroid hormone or indirectly by raising plasma calcium concentration. Whether "medical parathyroidectomy" achieved by these means reduces the need for surgical parathyroidectomy has yet to be formally demonstrated, but many investigators believe this to be the case.

The relationship between renal disease, osteomalacia and rickets, and deficiency of 1,25(OH)₂D₃ is not a clear one. Certainly, rickets in adolescents with renal failure shows a gratifying response to treatment with 1,25(OH)₂D₃. However, osteomalacia is an uncommon feature of renal bone disease in adults, being present in 10 to 20 per cent of patients, and does not respond particularly well to 1,25(OH)₂D₃ therapy. This mineralisation defect may depend much more upon the prevailing level of plasma phosphate, rather than on 1,25(OH)₂D₃. Furthermore, there is a group of patients with osteomalacia who are refractory to 1,25(OH)₂D₃ due to the toxic action of substances such as aluminium (Parkinson, Ward and Kerr 1981).

The major hazard of treatment is hypercalcaemia. Even with frequent measurement of plasma calcium, many patients become hypercalcaemic at some stage. There are two particularly vulnerable periods: at the beginning of treatment in those patients who start with a high plasma calcium level and who may ultimately respond poorly; and in patients who respond well, a tendency to become hypercalcaemic as their bone disease is cured.

Treatment of renal bone disease should not be undertaken without appropriate control of plasma phosphate with phosphate-binding agents, since intestinal absorption of phosphate is also increased and the combined increment in calcium and phosphate levels will favour extra-skeletal calcification (Parfitt 1969; Velentzas et al. 1978). For similar reasons these compounds should also be used cautiously, if at all, in patients with pre-existing hypercalcaemia. In addition, such patients often respond poorly to treatment and may require parathyroidectomy (Coburn et al. 1977; Kanis et al. 1977a) if hypercalcaemia is due to "autonomous" hyperparathyroidism. Where parathyroidectomy is planned, the pre-operative administration of vitamin D analogues for several weeks may decrease the risks of severe hypocalcaemia after operation which commonly occurs in patients with bone disease (Boyle et al. 1977; Parsons et al. 1977). Small doses should be used coupled with meticulous patient supervision to avoid toxicity. The efficacy of 1,25(OH)₂D₃ or 1α-OHD₃ used in this way has been recently challenged (Heath et al. 1979), but in our experience we have found this to be a useful therapeutic approach. Moreover, we have been occasionally surprised
to see favourable responses to medical treatment so that parathyroidectomy has been avoided.

A potential hazard of vitamin D treatment is the impairment of renal function in patients not yet with end-stage renal failure. Whereas there is little doubt that hyperphosphataemia and hypercalcaemia can jeopardise renal function, often irreversibly, the question is open whether or not renal failure is accelerated when toxicity is avoided (Tougaard et al. 1976; Christiansen et al. 1978; Kanis, Cundy and Naik 1978a; Naik et al. 1981).

There can be little doubt that these new derivatives are powerful and useful therapeutic tools in the management of renal bone disease. Although DHT, 1α-OHD₃ and 1,25(OH)₂D₃ have been used in patients without biochemical or radiographic evidence of bone disease, it is not yet known whether bone disease can be prevented. Whether they should be given prophylactically to prevent bone disease remains unclear, but in view of the potential hazards in their use my personal view is that they should be reserved for patients with demonstrable abnormalities.

**Hypoparathyroidism and pseudohypoparathyroidism.** The features of hypoparathyroidism include hypocalcaemia, hyperphosphataemia, intracranial calcification, impaired mental skeletal and dental development and symptoms from hypocalcaemia. Hypoparathyroidism may be idiopathic (often associated with other endocrine disturbances) or may follow surgical removal of the parathyroids. Occasionally patients having the biochemical features of hypoparathyroidism have normal or increased circulating levels of parathyroid hormone and it is thought that target-organ resistance to the actions of parathyroid hormone is responsible for the clinical and biochemical features—pseudohypoparathyroidism.

Parathyroid hormone has an important role in increasing renal tubular reabsorption of calcium and decreasing renal tubular reabsorption of phosphate; and lack of parathyroid hormone, or renal resistance to its action, largely accounts for the hyperphosphataemia and hypocalcaemia which are characteristic of hypoparathyroidism (Peacock, Robertson and Nordin 1969). Impaired production of 1,25(OH)₂D₃ due to hyperphosphataemia, may contribute to hypocalcaemia. Certainly 1,25(OH)₂D₃, DHT and 1α-OHD₃ can all rapidly reverse hypocalcaemia (Kooh et al. 1975; Russell et al. 1974). The rapid response to changing doses, within two to three days, offers a significant advance over other forms of treatment. If hypercalcaemia is inadvertently induced, the return to normal after stopping treatment is equally rapid.

The dose required to maintain normocalcaemia is probably less variable in patients with hypoparathyroidism than in patients with renal bone disease where requirements and tolerance often decrease with time (Fig. 7). However, fluctuations in dose requirements do occur (Parfitt and Frame 1972; Avioli 1974) which may be due to changes in dietary phosphate, immobilisation or other factors.

**Vitamin D-dependent rickets.** Before the advent of 1,25(OH)₂D₃ this form of inherited rickets was characterised by the usual biochemical and radiographic features of rickets, which would only respond to doses of vitamin D in the order of 10,000 units per day. These doses are lower than those required for classic vitamin D-resistant rickets but are much higher than those required to treat simple vitamin D deficiency. Similarly, partial resistance to treatment with 25-OHD₃ also occurs but responses are complete with small doses (0.5 to 2.0 micrograms per day) of 1,25(OH)₂D₃. This supports the idea that the disorder is due to a lack of renal 1α-hydroxylase activity and 1,25(OH)₂D₃ is therefore the treatment of choice in such patients. A subgroup of such patients has been found to have normal plasma levels of 1,25(OH)₂D₃—so-called Type II vitamin D-dependent rickets (Brooks et al. 1978). Such patients require somewhat higher doses (up to three micrograms daily) of 1α-OHD₃ or 1,25(OH)₂D₃ (Balsan et al. 1977).

**Vitamin D-resistant rickets.** The aetiology of rickets and osteomalacia in this heterogeneous group probably depends in part on the nature of the renal tubular defect. For example, in patients in whom renal tubular acidification is defective, systemic acidosis and phosphate depletion may both contribute to their bone disease. In those patients in whom the renal tubular defect is a failure to reabsorb normal amounts of phosphate, the hypophosphataemia per se rather than deficiency of 1,25(OH)₂D₃ produces the mineralisation defect. There is an X-linked inherited form of this disease but in our experience the sporadic form is more common. There are reports of successful treatment by phosphate supplements alone but most clinicians give large doses of vitamin D as well. It is now clear that relatively large doses of 1,25(OH)₂D₃, DHT or 1α-OHD₃ can substitute for vitamin D in the treatment of such patients (Saville et al. 1955; Brickman et al. 1973; Russell et al. 1975). However, again it is important to emphasise that this does not imply that the disease is due to deficiency of 1,25(OH)₂D₃.

**Osteoporosis.** There are now several studies indicating that small doses of 1,25(OH)₂D₃ or 1α-OHD₃ (0.5 to 2.0 micrograms per day) increase calcium absorption, as expected, in patients with osteoporosis. The therapeutic value of this change depends critically upon whether bone loss can be prevented or bone mass restored in such patients. Although calcium balance may become positive, long-term experience suggests that the effect is ill-sustained (Fig. 9; Christiansen et al. 1981).

Since many patients with osteoporosis may also have osteomalacia, the long-term effects on bone mineral still require careful evaluation. However, the hazards cannot be ignored. Hypercalcaemia is readily induced, particularly in the elderly who may have impaired renal function. Any wide scale use of 1,25(OH)₂D₃ will require extremely careful monitoring. Furthermore, the advantages of 1,25(OH)₂D₃ over other treatments such as fluoride or oestrogen remain to be demonstrated. It has
now been shown that oestrogen itself will stimulate endogenous 1,25(OH)₂D₃ production in patients with osteoporosis and many might argue that a safer mode of approaching treatment in such patients would be to use oestrogens alone or combined with very small amounts of vitamin D. By giving parent vitamin D rather than 1,25(OH)₂D₃, the production of endogenous 1,25(OH)₂D₃ is subject to the usual physiological restraints. However, not all patients can tolerate oestrogens and anxieties remain about their long-term hazards and the effects of their withdrawal.

Preparation for parathyroid surgery. Hypocalcaemia is a frequent complication after parathyroidectomy, and is especially severe in patients who have overt parathyroid bone disease (see chronic renal failure). Therefore, 1,25(OH)₂D₃ and 1α-OHD₃ have been used to prepare patients for parathyroid surgery and to manage the postoperative period.

Miscellaneous and future applications. Both 1,25(OH)₂D₃ and 1α-OHD₃ have been used in the management of neonatal hypocalcaemia and rickets and it is likely that they would be of value in the symptomatic treatment of hypocalcaemia of most causes. More work is needed to assess their value in other situations, for example, in patients on anticonvulsants who may be partially resistant to treatment with ordinary vitamin D. Similarly, 1,25(OH)₂D₃ may be of some value in treatment of the osteopenia associated with steroid therapy, diabetes and in the bone disease associated with hepatobiliary disease. There has been recent interest in the use of 1α-OHD₃ and 1,25(OH)₂D₃ in the treatment of skeletal disorders associated with various gut diseases including malabsorption syndromes and gastrointestinal bypass surgery. From the limited experience so far gained it appears that near physiological doses of 1α-OHD₃ given by mouth can improve osteomalacia whereas large oral doses of vitamin D₂ prove ineffective. Vitamin D is absorbed largely in fat and appears in the lymphatic circulation. It is probable that the more polar metabolites (1,25(OH)₂D₃, 1α-OHD₃ and DHT) are absorbed independently into the portal circulation. The preservation of this latter mechanism in bowel disorders might explain the efficacy of low doses of 1,25(OH)₂D₃ and 1α-OHD₃ in treating associated bone diseases. Veterinary applications include the treatment of milk fever or parturient hypocalcaemia.

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