In 1919 Sir Edward Mellanby produced experimental rickets for the first time and reported on its subsequent cure and prevention with cod liver oil. Within the next six years, Steenbock and associates discovered that antirachitic activity could be produced in animals and food by ultraviolet irradiation. During the 1920’s and 1930’s accelerated interest in this “antirachitic activity” led to the synthesis of the natural provitamin, 7-dehydrocholesterol, and ultimately to the isolation and identification of vitamin D [1]. In the last 20 years, vitamin D has been shown to play a pivotal role in the biochemical control of intestinal calcium absorption and bone remodeling. Of utmost significance was the discovery that vitamin D in itself was inert and that metabolic activation was essential to its biological expression. Today, in fact, the term “vitamin” D is a misnomer for all practical purposes since its endogenous production by the skin, distribution by the blood stream, the negative feedback control of its metabolism, and its mode of action through the stimulation of protein synthesis, all resemble hormonal rather than dietary vitamin activity.

Intermediary metabolism of vitamin D. Within the last decade, considerable knowledge has accumulated regarding the metabolism of vitamin D and its biological activity in health and disease. In man, ultraviolet irradiation isomerizes 7-dehydrocholesterol in skin to vitamin D₃ resulting in an average vitamin D₃ content of approximately 3.2 µg/g tissue or 17 IU/cm² of skin [2]. The preformed vitamin D₃ is then absorbed into the micro-circulation and transported in plasma while bound to a specific globulin of d > 1.21 and a molecular weight of approximately 60,000 [3, 4]. Dietary vitamin D₂ (ergocalciferol) or vitamin D₃ (cholecalciferol) are absorbed bound to lipoproteins primarily from the duodenum and jejunum into lymphatic channels with both bile salts as well as intestinal lipids permissive in this regard [5]. The absorbed vitamin D₂ or D₃ then admixes with endogenously synthesized vitamin D₃ and is either sequestered in storage depots or metabolically transformed. Adipose tissue and muscle appear to be major storage sites for vitamin D₃ [6, 7], presumably serving as a source of the vitamin during dietary deprivation and/or lack of sunlight exposure. Circulating vitamin D₃ levels, as determined by gas-liquid chromatographic techniques [8] in subjects on routine diets, normally range between 8 and 45 ng/ml; at this plasma level, vitamin D₃ disappears with a biological half-time of 19 to 25 hr [3, 9]. Endogenously synthesized vitamin D₃ (and presumably vitamin D₂ as well) are metabolized to their respective 25-hydroxylated derivatives (i.e., 25-hydroxycholecalciferol and 25-hydroxyergocalciferol) by a hepatic mitochondrial enzyme system which requires reduced pyridine nucleotide and molecular oxygen [10]. Although it has been established that the 25-hydroxylation conversion system in the liver is feedback-regulated by the product of its action, the exact nature of this servo-control mechanism is still poorly understood, as is the role it plays in preventing the toxic effects of vitamin D overdosage. The hydroxylated vitamin D metabolites 25-hydroxycholecalciferol (25HCC) and 25-hydroxyergocalciferol (25HEC), subsequently circulate bound to a alpha globular protein indistinguishable from that which binds the parent vitamin D, with a biological half-time of 13 to 19 days [3]. A recently developed competitive protein-binding radio-ligand assay allows precise determination of the 25-hydroxylated vitamin D derivatives [11, 12]. Unfortunately the method does not yet distinguish 25HCC from 25HEC. Combined 25HCC-25HEC levels in normal subjects, as quantitated by this technique, average 20.5 ± 3.0 ng/ml [11]; a direct correlation exists between individual 25HCC-25HEC values, vitamin D intake, exposure to sunlight and total serum calcium values. 25-hydroxylated forms of vitamin D represent the major circulating vitamin D metabolites and a direct action of 25HCC has been demonstrated on bone [13] and intestine [14]. It has also been shown that 25HCC increases the proximal tubular reabsorption of phosphate, and that this activity is antagonistic to the phosphaturic effect of parathyroid hormone [15].

Whereas the metabolic fates of 25HCC in intestine and bone are still poorly defined, it is further hydroxylated to either 1,25-dihydroxycholecalciferol (1,25DCC) or 24,25-dihydroxycholecalciferol1 (24,25DCC) by a carbon monoxide-sensitive mitochondrial system which also requires reduced pyridine nucleotide and molecular oxygen for maximal activity [16, 17]. Preliminary observations are consistent with the view that the conversion of 25HCC to either 1,25DCC or 24,25DCC is regulated by a second servo-control mechanism modulated by circulating calcium,

1 Recent observations suggest that the second hydroxyl group is in position “24” in this metabolite rather than position “21” as previously reported.
parathyroid hormone and calcitonin [18–21]. Parathyroid hormone stimulates 1-hydroxylation of 25HCC to 1,25DHCC whereas calcitonin promotes 24-hydroxylation of 25HCC to 24,25DHCC. In defense of this hypothesis are the observations that thyroparathyroidectomized animals do not synthesize 1,25DHCC and that exogenous administration of parathyroid hormone restores the renal synthesis of 1,25DHCC to normal [20]. In addition, whereas circulating 24,25DHCC is undetectable in the presence of parathyroid tissue, it has been isolated from the plasma of a hypoparathyroid patient with known "resistance" to vitamin D therapy [22]. The current prevailing hypothesis is one which considers hypocalcemia as a stimulus for parathyroid hormone secretion, the latter stimulating the renal synthesis of 1,25DHCC which in turn completes the negative feedback loop system of hormonal control by stimulating the intestinal absorption and bone mobilization of calcium (Fig. 1). In contrast, hypercalcemia, while decreasing the rate of parathyroid hormone release, also stimulates calcitonin release, the combination resulting in decreased 1,25DHCC production, a stimulated production of 24,25DHCC and a decrease in both calcium absorption and bone mobilization [21, 23]. Although the metabolic fate of both 1,25DHCC and 24,25DHCC is still unknown, accumulated evidence is consistent with the view that 1,25DHCC is "the" vitamin D₃ metabolite which governs intestinal calcium transport and bone resorption.

1,25DHCC is four to thirteen times as effective as vitamin D₃ and over two times as effective as 25HCC in stimulating intestinal calcium transport [25].

The manner in which vitamin D metabolites exert control over calcium uptake and its transcellular migration is purely conjectural. Preliminary observations suggest that the diol or triol metabolites are initially bound to specific cytosol proteins [26] prior to their incorporation onto nuclear receptor sites where they ultimately affect either the transcriptional process directly [27, 28] or regulate the release or transport of nuclear-derived RNA. The final event in the sequence is the generation of proteins essential for calcium transport such as calcium-binding protein(s), alkaline phosphatase(s) and ATPase(s) [29]. It has been noted by some investigators that 1,25DHCC but not 25HCC induces calcium transport in rats previously treated with actinomycin D [30, 31]. These findings suggest that 1,25DHCC acts in the intestine by a process which does not involve the retrieval of genetic information and that the actinomycin D-sensitive component in the stimulated intestinal response to vitamin D is the conversion of 25HCC to 1,25DHCC in the kidney.² The fact that actinomycin D fails to inhibit the intestinal effect of 1,25DHCC does not

² Studies with isolated tubules are not in accord with this theory since parathyroid hormone and extracellular calcium ion have no regulatory effect on the renal tubular consumption of 25HCC or production of 1,25 DHCC [61].
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migrate against the theory that the action of vitamin D on the intestinal is mediated ultimately by transport proteins or enzymes. The vitamin may still function in this regard by activating certain protein precursors within the intestinal cell. Unlike 25HCC, 1,25 DHCC has a minimal effect on the renal tubular reabsorption of phosphate. However, 1,25 DHCC does possess bone resorptive properties with an in vitro effectiveness some 100 times that of the parent 25HCC [32]. The preferential accumulation of 1,25 DHCC in the nucleus of bone cells and the subcellular skeletal distribution pattern of other vitamin D₃ metabolites [33] suggest a mechanism of action for 1,25 DHCC in bone that resembles its influence on calcium translocation in the intestinal cell [27, 28]. Finally, although another triol metabolite of vitamin D (25,26-dihydroxycholecalciferol) with weak biological activity limited to the intestine has been isolated from plasma [34], its origin and metabolic fate has yet to be determined. On the basis of observations in man and a variety of animal species, the current working hypothesis depicts vitamin D₃ primarily as an inert storage substance, 25HCC as the major circulating metabolite and 1,25 DHCC as the ultimate biologically active metabolite; in concert with parathyroid hormone and calcitonin, they control the intestinal absorption of calcium and the remodeling of bone.

Vitamin D and chronic renal failure. Chronic renal failure is attended by an acquired impairment of intestinal calcium absorption, secondary hyperparathyroidism, and defective maturation of both osteoid and mineraliications of skeletal tissue [35—40]. Although some of these derangements in mineral metabolism are occasionally reversed temporarily by chronic hemodialysis they are usually refractory to dialytic therapy and may actually progress in an accelerated fashion during treatment. Disordered mineral metabolism in renal failure results in osteopenia, fracture, ectopic calcification and sustained hyperparathyroidism, all of which present a frustrating therapeutic challenge. So-called “renal osteodystrophy” subsequently becomes resistant to vitamin D therapy in that the requirement of the vitamin to produce an effective sustained biological response is increased when compared to dosages used in other nonuremic malabsorbive or demineralizing pathological states. Although the temporal relationship between the acquired resistance to vitamin D and advancing uremia is still ill-defined, it becomes evident when filtration rate is reduced to 15 to 20 ml/min.

It has been demonstrated with biological assay techniques that vitamin D activity (i.e., antirichitic activity) in the serum of untreated patients with chronic uremia is lower than that observed in either a well-fed healthy population [41, 42] or in malnourished individuals with terminal cachexia [42]. In addition, the patient with terminal uremia requires a greater intake of vitamin D and higher levels of assayable anti-richitic activity for biological effectiveness than does the normal individual [41]. This well-recognized phenomenon of vitamin D resistance in renal failure has been variably attributed to uremic toxins interfering with the normal conversion of vitamin D or its more active metabolites or the response of the end organs to these metabolites. Recent studies with radioisotopic forms of vitamin D₃ and 25HCC in subjects with renal failure [43, 44], in anephric animals [17, 45], and in animals with experimentally induced chronic renal failure [46], have focused attention on the loss of kidney cells rather than on the loss of renal excretory function as being responsible for the defect in intestinal calcium transport activity. All things being equal, one would anticipate that a reduction in kidney mass would result in decreased renal production of 1,25 DHCC. It also seems logical to assume that the subsequent reduction in the intestinal mucosal concentration of 1,25 DHCC leads to progressive decrements in calcium absorption. Results demonstrating that small doses of 1,25 DHCC augment calcium absorption and raise the serum calcium of uremic man are consistent with this hypothesis [47], as is the frequent failure of hemodialysis to reverse the progression of calcium malabsorption, renal osteodystrophy and vitamin D resistance.

Alterations in the metabolic fate of 25HCC, albeit well documented in uremic man and animals with experimental renal failure, do not as yet completely explain the calcium malabsorption of uremia nor a variety of other independent observations. Defects in calcium absorption are noted in experimental renal insufficiency at a time when the metabolism of vitamin D and the tissue localization of its metabolites are normal [48]. The possibility obtains that one or more uremic toxins may impose certain conditional alterations in the orderly sequence of intestinal cell multiplication, morphologic maturation, and in the translational and transcriptional processes normally regulating intestinal protein synthesis [49]. These vitamin D-independent changes might result in structural or catalytic defects in cellular protein components of the calcium transport system such as the calcium-binding protein(s) and ATPase(s), and consequently limit the translocation of dietary calcium across the intestinal cell. Since the uptake and release of calcium by intestinal mitochondria is fundamental to its cellular transport [29], the documented distortion of intestinal mitochondrial metabolism in uremia [50] may add to the sluggish migration of calcium through the mucosal cell. Since plasma levels of 25HCC-25 HEC do increase in uremic subjects during vitamin D therapy it has been assumed that hepatic 25-hydroxylation proceeds normally. However, patients on chronic hemodialysis can be separated into those with normal circulating 25HCC-25 HEC levels and ostitis fibrosa cystica and others with low 25HCC-25 HEC levels and osteomalacia [51] and rats with experimental renal insufficiency of five to nine weeks

3 Date in defense of this hypothesis are considerable but presently inconsistent with observations that complete inhibition of RNA synthesis by actinomycin D or protein synthesis by puromycin does not diminish 25HCC consumption or 1,25 DHCC production by intact renal tubule [62].
duration have lower circulating 25 HCC levels than their pair-fed controls [39]. In addition, it has been demonstrated that vitamin D therapy alone reverses the osteomalacia of bilaterally nephrectomized patients on hemodialysis [52] and that 25 HCC, in doses of 6,000 to 12,000 IU/day is effective therapy for uremic bone disease in individuals refractory to vitamin D in equivalent dosage [53]. These findings suggest that in end-stage renal failure, refractoriness to vitamin D may involve end-organ failure (i.e., mitochondrial metabolism or protein synthesis) secondary to a variety of uremic toxin(s). Alterations in the metabolism of vitamin D or its hydroxylated metabolites may also result in the accumulation of structurally related abnormal metabolites which compete with 1,25 DHCC for receptor sites in the nuclei of specific target organs. Alternatively, the possibility exists that tissue 1,25 DHCC receptor sites exhibit a relative rather than an absolute specificity for diol and triol vitamin D-related steroids and as such are readily available to vitamin D₃ and 25 HCC as well. Demonstrated in vitro effectiveness of vitamin D₃, dihydrotachysterol and 25 HCC on intestinal calcium absorption is compatible with this hypothesis [54]. It may also be premature to assume that negative feedback regulatory mechanisms for 25 HCC and 1,25 DHCC generation by the liver and kidney respectively (Fig. 1) are intact in the uremic subject. Although in theory, the attendant hypocalcemia and resultant hyperparathyroidism should compensate for the decrease in 1,25 DHCC production anticipated by the loss of renal mass, this does not appear to be the case since 1,25 DHCC production is decreased in animals and humans with terminal renal failure and secondary hyperparathyroidism. This relative inefficiency of the renal servo-control mechanism for 1,25 DHCC production may represent another example of end-organ resistance to parathyroid hormone often complicating the uremic syndrome [37].

Accumulated knowledge in the intermediary metabolism of vitamin D₃ and the defects in this pathway in chronic renal failure should lend itself to a more rational approach to the uremic patient. For example, the procedure of bilaterally nephrectomizing patients undergoing dialysis and programmed for renal transplantation should be tempered by the consideration that nephrectomy will remove those residual renal cells which are capable of synthesizing 1,25 DHCC and may ultimately lead to a progressive renal osteodystrophy which may be quite refractory to vitamin D therapy. Although 25 HCC [53, 55] or 1,25 HCC [49] may prove to be effective therapeutic agents, the supply of the former is quite limited and the synthesis of 1,25 DHCC too expensive to make its routine availability practical. In addition, both 25 HCC and 1,25 DHCC may be inappropriate therapeutic agents in chronic renal failure since their ability to activate intestinal calcium absorptive mechanisms is most probably accompanied by an exaggeration of the already increased bone resorption. Thus the reported hypercalcemic-hyperphos-

Fig. 2. Structural similarities between 25-hydroxycholecalciferol (25HCC), 1,25-dihydroxycholecalciferol (1,25DHCC), 25-hydroxydihydrotachysterol₃ (25H DHT₃) and 5,6-trans-25-hydroxycholecalciferol (5,6-trans-25HCC).
substantially from vitamin D. However, such a closely related compound might be able to overcome strict stereochemical constraints by mass action. This theory is also compatible with the observed structural specificities for both the intestinal and bone receptor sites for a variety of biologically active "vitamin D₃-like" compounds. Those with side chains of vitamin D₃ or dihydrotrachysterol act preferentially on the intestine whereas compounds with the side chain of vitamin D₂ (ergocalciferol) or dihydrotrachysterol act preferentially to mobilize skeletal mineral [59].

The ideal drug of choice in the management of the bone disease of renal failure is one which not only stimulates the intestinal absorption of calcium with the effectiveness of 1,25 DHCC but one which is also devoid of the bone mobilizing effect of this triol metabolite of vitamin D₃. Recent attempts to develop a compound with these unique properties have led to the synthesis of the 5,6-trans isomer of 25HCC. Compared to 25HCC, the 5,6-trans isomer has its A-ring rotated 180°, with the 3β-hydroxyl group in the same geometrical position as the 1-hydroxyl of 1,25 DHCC (Fig. 2). Since 5,6-trans 25HCC reportedly is more active than 25HCC in stimulating intestinal transport in the anephric state and demonstrates little if any potential to induce bone resorption in vitro [60], it becomes one of the most promising drugs for the simultaneous reversal of the calcium malabsorption and the osteodystrophy associated with uremia. The knowledge derived from continued investigation with 5,6-trans 25HCC and structurally related synthetic analogues in acute anephric or chronic uremic states should add considerably to existing concepts on the specific function of individual biologically active vitamin D metabolites and their modes of action on kidney, intestine and bone.

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