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Vitamin D metabolism

Recent advances

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Knowledge of the mode of action and metabolism of vitamin D has made rapid progress in the past few years. The discovery that ingested vitamin D undergoes transformation to a circulating metabolite, that this metabolite then undergoes further transformation into a more biologically active form, and that production of these metabolites may be subject to feedback control mechanisms, has led to the recognition that 'vitamin D' could be regarded as a hormone as well as a vitamin. This hormonal concept of the antirickets agent is far from new, however, for in 1919, the same year that Mellanby discovered that cod liver oil could cure rickets, Huldschinsky (quoted by Loomis, 1970) showed that ultraviolet irradiation of a single limb would cure rickets not only in the irradiated limb but in the other limbs as well; these findings had thus already fulfilled the criteria of a hormone as a substance synthesized at one site in the body and exerting a powerful metabolic effect at a site distant from the point of synthesis. The nature of this effect has now become clearer, and the purpose of the present review is to trace the most recent developments in this field and their bearing on problems in clinical medicine.

Much of our knowledge of the physiology of vitamin D is summarized in Fig 1. Cholecalciferol (vitamin D₃) and possibly other antirachitic compounds are formed in the skin by the action of ultraviolet irradiation on the physiological precursor 7-dehydrocholesterol. 7-Dehydrocholesterol synthesis is active in both liver and skin (Gaylor and Sault, 1964), but the exact way in which its irradiation products are absorbed through the skin in man is uncertain. Early studies (Helmer and Jansen, 1937a, b) showed that antirachitic material was recoverable from human skin washings and that cholecalciferol could cure rickets when applied to unbroken animal skin. More recently Gaylor and Sault (1964) observed that the concentration

of 7-dehydrocholesterol was maximal in the dead keratin and sebaceous gland fractions of rat skin. It is therefore possible that 7-dehydrocholesterol may be secreted by sebaceous glands onto the skin surface, converted there by ultraviolet irradiation, and then reabsorbed. Such a physiological process might thus incriminate too frequent skin washing, for example in accordance with certain religious

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FIG. 1.—Major pathways of vitamin D metabolism in man. Pathways are represented through skin, liver, small intestine, kidney, bone, and storage reservoir of muscle and adipose tissue. 25-HCC = 25-hydroxycholecalciferol, 1,25-DHCC = 1,25-dihydroxycholecalciferol, CaBP = calcium-binding protein.

principles, as an additional factor in the production of rickets and osteomalacia. Evidence is conflicting, however, since data of Wheatley and Reinertson (1958) suggested that 7-dehydrocholesterol was maximally concentrated in the malpighian layer of human skin and that very little was present in the dermis where the sebaceous glands arise. Furthermore, Thomson (1955) showed that a large proportion of ultraviolet light of wavelength which included the antirachitic range (290–320 m μ) could readily penetrate the stratum corneum of Caucasian skin (Loomis, 1967). There may thus be no need to postulate that 7-dehydrocholesterol must first reach the skin surface before effective irradiation occurs, as in fur-bearing animals. While further work on skin synthesis and absorption of cholecalciferol is required, it is almost certain that dietary sources of vitamin D are only required when a man is shielded from effective sunshine by clothing, housing conditions, or industrial smog.

Apart from certain oily fish which contain large quantities of cholecalciferol (which they synthesize for reasons which are at present obscure), natural animal products are surprisingly deficient in vitamin D considering the natural connotations of the word 'vitamin'. Fig. 1 shows vitamin D₃ (and calcium salts) from natural dietary sources entering the small intestine. Vitamin D₃ is absorbed throughout the small intestine in humans, though the site of maximal absorption is still uncertain (Arnstein, Frame, and Frost, 1967). Bile salts appear absolutely necessary for its absorption in micelle form (Schachter, Finkelstein, and Kowarski, 1964; Avioli, 1969), and hepatic osteomalacia due to biliary obstruction can be cured by parenteral administration of physiological amounts of cholecalciferol (Dent and Stamp, 1970). Nevertheless, osteomalacia remains an uncommon complication of long-standing liver disease, and osteoporosis alone is much more commonly seen (Atkinson, Nordin, and Sherlock, 1956). Almost any form of steatorrhoea may be complicated by rickets or osteomalacia, and when it is due to gluten-sensitive enteropathy the rickets may be resistant even to quite large doses of vitamin D injected parenterally (Nassim *et al.*, 1959). This is considered further below. The osteomalacia which may follow partial gastrectomy may not infrequently be due to self-restriction of vitamin D-containing fatty foods (Thompson, Lewis, and Booth, 1966).

Major advances in our understanding of vitamin D metabolism have followed the use of isotopically labelled cholecalciferol. The first studies in this field were performed by Kodicek (1955) but it was

not until 11 years later that Neville and DeLuca (1966) and Callow, Kodicek, and Thompson (1966) were able to synthesize an isotope of high enough specific activity (20,000–26,000 DPM/IU) to clarify the pathways of vitamin D metabolism. After administration of the isotope to intact animals, chromatographic techniques were used to separate further discrete peaks of radioactivity corresponding to hydroxylated, and therefore more polar, metabolites of the parent vitamin. It was then shown that the major circulating form of the vitamin was a compound in which a second hydroxyl group had been introduced, and this was identified by DeLuca and his coworkers (DeLuca, 1969) as 25-hydroxycholecalciferol (25-HCC, Fig. 2). It was shown that synthesis of 25-HCC could be accomplished only by liver (Ponchon and DeLuca, 1969), and more recently this synthesis has been shown to be product-inhibited (DeLuca, 1971). There is also an enterohepatic recirculation of vitamin D and its metabolites, largely conjugated as glucuronides before secretion into the bile (Avioli *et al.*, 1967), and bile fistulae may thus lead to vitamin D depletion. Earlier studies by Zull, Czarnowska-Misztal, and DeLuca (1966) had shown that after oral administration of cholecalciferol to rachitic rats there was a 16- to 20-hour delay before increased calcium uptake by subsequently-removed small intestine could be shown *in vitro*. This time lag was reduced to about 6 hours when 25-HCC instead of cholecalciferol was administered, but the continuing, though smaller, time lag suggested that further metabolism to a compound with immediate biological activity might be required. Lawson, Wilson, and Kodicek (1969) had detected a previously undescribed metabolite in isolated chick intestinal cell nuclei, and it was further discovered that this metabolite could be synthesized only by kidney (Fraser and Kodicek, 1970). This metabolite, identified as 1,25-dihydroxycholecalciferol (Fig. 2, 1,25-DHCC) by Lawson *et al.* (1971), was found to have a powerful action in promoting intestinal calcium absorption (Kodicek, Lawson, and Wilson, 1970). Its structure and immediate powerful effect have been amply confirmed (Holick, Schnoes, and DeLuca, 1971; Haussler *et al.*, 1971; Myrtle and Norman, 1971; Wong and Norman, 1972), and 1,25-DHCC is now regarded as the active, hormonal form of the vitamin. Other polar metabolites of cholecalciferol have also been isolated, including 25,26-dihydroxycholecalciferol with some action on intestine but none on bone, and one, whose characterization has become uncertain, which has some action on bone but little on intestine (Suda *et al.*, 1970b, a). The pos-

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FIG. 2.—Chemical transformations of vitamin D₂ and D₃ and their major metabolites. Ergosterol differs only in its side chain from 7-dehydrocholesterol and only this side chain is shown. The same changes are produced in both molecules by UV irradiation. Only the side chain of 25-hydroxycholecalciferol (25-HCC) is represented. Only the lower rings (A-rings) of 1,25-dihydroxycholecalciferol (1,25-DHCC) and of dihydrotachysterol (DHT) are shown.

sible physiological or clinical significance of these compounds is not understood, but one of them, now thought to be 24,25-dihydroxycholecalciferol, may be synthesized by kidney in inverse proportion to 1,25-DHCC under the influence of certain controlling factors. The accumulation of 1,25-DHCC in the intestine and other organs was found to be regulated in part by plasma calcium concentrations (Boyle, Gray, and DeLuca, 1971). Thus low calcium intake and associated hypocalcaemia in vitamin D-deficient rats resulted in the rapid accumulation of 1,25-DHCC in the intestine and other organs. As dietary, and therefore plasma, calcium levels were raised, the accumulation of 1,25-DHCC decreased while the proportion of the second metabolite rose (Omdahl *et al.*, 1972). It has now been established that this control of synthesis of 1,25-DHCC is effected by the parathyroid gland. Thus, in the experimental animal parathyroidectomy diminishes 1,25-DHCC synthesis and the administration of parathyroid extract restores it (Garabedian *et al.*, 1972), while opposite changes occur in synthesis of the other renal metabolite. Stimulation of 1,25-DHCC synthesis by parathyroid hormone (and by cyclic AMP as well) has been shown in isolated renal tubules (Rasmussen *et al.*, 1972), and corresponding changes in the responsible renal enzyme activity have also been shown (Fraser and Kodicek, 1973).

Thus a further refinement in calcium homeostasis may be described in which parathyroid hormone secretion, responding to a fall in plasma calcium, not only influences bone calcium mobilization directly but also stimulates 1,25-DHCC synthesis. This in turn enhances both intestinal calcium absorption and bone calcium mobilization (see below), the process being reversed as plasma calcium rises.

Synthesis of 1,25-DHCC by the kidney and its subsequent localization in the intestine is represented in Fig. 1. Calcium is absorbed from the intestinal lumen and transported across the mucosal cell in association with calcium-binding protein (CaBP). The precise mechanism of CaBP production is also uncertain; DNA transcription into messenger RNA is required for the renal synthesis of 1,25-DHCC; actinomycin D, therefore, inhibits this step; the drug has been claimed, however, not to affect the formation of CaBP, under the influence of 1,25-DHCC, from a possible precursor protein in the intestine (Drescher and DeLuca, 1971; Corradino and Wasserman, 1972), but evidence here is at present conflicting (D. E. M. Lawson, personal communication, 1972). Absorbed calcium is then transported to the osteoid seams and growing cartilage of bone where it is laid down with phosphate as hydroxyapatite in calcifying tissue.

A possible physiological requirement for vitamin D or its derivatives for normal calcification in

bone is still uncertain. The gross histological abnormality of osteomalacic bone with its overall increase in osteoid tissue has suggested to many that vitamin D may play a further part in normal calcification in addition to ensuring an adequate calcium $\times$ phosphorus solubility product in the blood. However, *in vitro* studies have so far shown that certain vitamin D metabolites produce only increased bone resorption. Cholecalciferol was inactive in these systems, 25-HCC and parathyroid hormone (also heparin and vitamin A) caused increased resorption (Reynolds, 1972), while 1,25-DHCC was 100-fold times more active in this respect (Raisz *et al.*, 1972). However, in the experimental animal, nephrectomy completely prevents the bone calcium mobilization response to 25-HCC, but not to 1,25-DHCC (Holick, Garabedian, and DeLuca, 1972a). These findings are thus consistent only with the clinical effects of vitamin D intoxication and to date do not implicate a direct role of vitamin D in normal calcification. The chemical formulae of cholecalciferol and the yeast vitamin ergocalciferol (vitamin D₂), together with their major metabolites, are shown in Fig. 2.

Synthesis of 1,25-DHCC in human kidney has been confirmed (D. R. Fraser, personal communication, 1971) and characteristic radioactivity corresponding to 1,25-DHCC has been detected in human plasma (Mawer *et al.*, 1971a; T. C. B. Stamp and D. E. M. Lawson, unpublished data), but not in a nephrectomized patient (Mawer *et al.*, 1971a). Its renal synthesis in rats has recently been localized to the tubule (Shain, 1972). Failure of the renal synthesis of 1,25-DHCC thus provides the most logical explanation of much of the mystery of renal rickets, and of the early occurrence of rickets in patients with severe tubular failure but little glomerular failure as in the Lignac-Fanconi syndrome (cystinosis) with its early characteristic 'swan-neck' deformity of the proximal renal tubule. Atrophy of the jejunal mucosa with consequent failure of the 1,25-DHCC/calcium-binding protein system may provide part of the explanation for vitamin D resistance in gluten-sensitive enteropathy.

The liver of man and animals has long been considered as the major storage site of vitamin D, and indeed animal liver is the only meat product that contains significant amounts. Nevertheless, necropsy and amputation studies in patients who had received pharmacological amounts of vitamin D (Lumb, Mawer, and Stanbury, 1971) showed with bioassay techniques that skeletal muscle and adipose tissue may provide a large storage reservoir from which vitamin D may be slowly released as plasma levels fall. The form in which vitamin D

is stored in this reservoir, whether as the parent vitamin or as 25-HCC, is uncertain, since chemical rather than bioassay analysis must be applied.

Various therapeutic trials of 25-HCC in pharmacological dosage have been reported in the sex-linked form of vitamin D-resistant rickets, in hypoparathyroidism, anticonvulsant osteomalacia, and in chronic renal failure, and recent evidence suggests that in some situations it may be several times more potent on a weight basis than the standard preparations of vitamin D₂ or D₃ in current use (Balsan and Garabedian, 1972; Stamp *et al.*, 1972). Further strides in this field have been made with the recent development of competitive protein-binding assays for 25-HCC, which have permitted its accurate chemical determination in human plasma (Belsey, DeLuca, and Potts, 1971; Haddad and Chyu, 1971). Mean 25-HCC levels in both normal adults and adolescent school-children in London were 16 ± 1 (SEM) ng/ml (Stamp *et al.*, 1972). On an equivalence of 1 mg 25-HCC = 50,000 IU of vitamin D activity (DeLuca, 1971), this corresponds to a figure of 0.8 IU/ml; this same figure for normal subjects has been reported by Lumb *et al.* (1971) in Manchester, Thompson *et al.* (1967) in London, and Illig, Antener, and Prader (1961) in Switzerland using bioassay techniques. The possibility that circulating antirachitic activity may thus be composed largely if not entirely of 25-HCC in normal human plasma is consistent with studies using isotopically labelled cholecalciferol which showed the virtual disappearance of isotopically labelled D₃ from the plasma within 2 to 5 days after injection (Mawer *et al.*, 1971b); unpublished studies in our own laboratory have confirmed this. The actual rate of disappearance of isotopically labelled cholecalciferol and rate of appearance of 25-HCC and other more polar metabolites has been shown to depend upon pool size in individual patients, that is on their state of vitamin D nutrition (Mawer *et al.*, 1971b), rather than reflecting any abnormality of vitamin D metabolism as was first proposed by Avioli and his co-workers (Avioli *et al.*, 1968) for patients with chronic renal failure.

25-hydroxylation of cholecalciferol and of ergocalciferol proceeds normally in patients with both sex-linked variety of hypophosphataemic rickets and the recessively-inherited vitamin D-dependency rickets (Haddad *et al.*, 1973). The occurrence of rickets and osteomalacia due to long-term anticonvulsant therapy in epilepsy (Kruse, 1968; Dent *et al.*, 1970) was postulated to arise from hepatic enzyme induction leading to increased breakdown of vitamin D to inactive metabolites

and thereby to increased vitamin D requirement in these patients. Confirmation of this theory has been assisted by the demonstration of abnormally low levels of 25-HCC in plasma from these patients (Stamp *et al.*, 1972) and by the demonstration of phenobarbitone-induced alterations in vitamin D metabolism (Hahn *et al.*, 1972). Other forms of hereditary or acquired vitamin D-resistant rickets (Dent, 1970) may well result from abnormalities of vitamin D metabolism, and clinical scientists are now poised to explore and clarify many of these poorly understood conditions. Meanwhile, many old established clinicopharmacological phenomena are already clearer. The delayed action of vitamin D₂, when given in large doses to heal metabolic rickets or to raise the plasma calcium in hypoparathyroidism, is clearly due to its required conversion to a more active form. This conversion may also be severely inhibited by the lack of parathyroid hormone. The rapid action of dihydrotachysterol (DHT, AT 10) in raising plasma calcium may result from its steric configuration which in some ways more closely resembles 1,25-DHCC (Fig. 2). This resemblance could give it a rapid action on bone resorption in hypoparathyroidism and, in high dosage, on the CaBP system in various forms of resistant rickets. On the other hand the inadequate conversion of DHT to a true hormonal form of the vitamin, such as 1,25-DHCC or 1,25-dihydroxy-ergocalciferol, may explain its inadequacy in small dosage in the treatment of nutritional rickets.

Synthetic analogues of vitamin D₃ and 25-HCC in which the A-ring has been rotated through 180° so that it resembles the steric configuration of DHT in the position of the hydroxyl group (Fig. 2) have recently been found effective on bone and intestine in the absence of the kidneys, a situation where the natural compounds are without effect (Holick, Garabedian, and DeLuca, 1972b). The well-founded fear that most clinicians have of the occasional unpredictable effects of high-dosage vitamin D therapy, ranging from inactivity to sudden inexplicable intoxication may be related to various steps in its metabolic feed-back control. The pathways of vitamin D metabolism are thus affected by many at first seemingly unrelated extraneous factors which range from renal failure through intestinal mucous membrane atrophy to anticonvulsant therapy, and a host of inborn errors of metabolism. Our accumulating knowledge is helping to explain much of this, and as the vitamin D metabolites themselves and their analogues become available for therapy, both powerful and

safer new therapeutic weapons should lessen the worries of the practising clinician.

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