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INTESTINAL CALCIUM ABSORPTION *and its* REGULATION

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PREFACE

This review will encompass the gastrointestinal absorption of calcium and its regulation. The importance of calcium in biological systems is unquestionable. Its role in maintaining the integrity of cell membranes and normal function in muscle, nerve, and secretory organs is well known. It is not surprising that the calcium level in plasma and extracellular fluid is sustained within very narrow limits by homeostatic mechanisms. These mechanisms, at least in mammals, involve three endocrine messengers: parathyroid hormone, calcitonin, and 1,25-dihydroxyvitamin D_3. Three organs — kidneys, gut, and bone — are the targets of these hormones.

The decade since 1970 has seen tremendous advances in our understanding of the physiological role of vitamin D. These developments have focused on the hormonal function of vitamin D_3 with the recognition of the role of the renal-vitamin D endocrine system.

In the review to follow emphasis will be placed on: (1) the recent developments in the metabolism, transport, and endocrine function of vitamin D; (2) the concepts and techniques concerning calcium transport by the gastrointestinal tract; and (3) the physiological and pharmacological aspects of gastrointestinal absorption of calcium.

AUTHOR

Dr. Alexander D. Kenny obtained his Bachelor of Science degree in Chemistry at the Imperial College of Science and Technology, University of London, in 1945. He obtained his Ph.D. degree in Biochemistry at the Institutum Divi Thomae, Athenaeum of Ohio, in 1950. After receiving postdoctoral training in endocrinology with Dr. Paul L. Munson at the Harvard School of Dental Medicine, he has held academic appointments in Pharmacology at Harvard Medical School, West Virginia University, University of Missouri (Pharmacology and Biochemistry), and The University of Texas Medical Branch in Galveston. In 1976, Dr. Kenny was appointed Professor and Chairman of the Department of Pharmacology and Therapeutics and Director of the Tarbox Parkinson's Disease Institute at the Texas Tech University Health Sciences Center in Lubbock. Dr. Kenny has also held appointments in the Medical Unit, University College Hospital in London, at the Massachusetts General Hospital, and at the Sidney Farber Cancer Institute in Boston during the 1950s. It was during his association with the late Professor Charles E. Dent, M.D., F.R.S. at University College Hospital in London in 1950–51 that Dr. Kenny first became interested in the endocrine aspects of calcium metabolism.

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Part I
Vitamin D Metabolism

INTRODUCTION

Rapid developments in our understanding of vitamin D metabolism took place in the three-year span 1968 to 1971. It was during this period that important contributions from the work of Kodicek, DeLuca, Norman, Haussler, and their respective associates led to our recognition of the renal-vitamin D endocrine system.[1] The early reports indiated that vitamin D_3 was converted to a more polar metabolite which was identified as 25-hydroxyvitamin D_3[2] and that the liver was the site of this conversion.[3] A second metabolite, subsequently known as 1,25-dihydroxyvitamin D_3, was detected in avian intestinal nuclear chromatin and was proposed as the active form of vitamin D_3.[4] This metabolite was subsequently shown to be formed exclusively in the kidney[5] and identified as 1,25-dihydroxyvitamin D_3.[6,7] It is now accepted that vitamin D_3 and its liver metabolite, 25-hydroxyvitamin D_3, are physiologically inactive precursors or prohormones of 1,25-dihydroxyvitamin D_3, the active form of the vitamin. The latter is now designated a hormone secreted by its endocrine organ, the kidney. The overall scheme describing our present knowledge concerning vitamin D metabolism is presented in Figure 1. Plasma concentrations of vitamin D and its major metabolites are presented in Table 1, Chapter 2. The major circulating form of vitamin D is 25-hydroxyvitamin D (30 ng/mℓ); vitamin D itself is much lower (1 ng/mℓ). The plasma concentration of the hormonal form, 1,25-hydroxyvitamin D, is considerably less (0.03 ng/mℓ).

In the more detailed discussion to follow, attention will be focused on four major areas of vitamin D metabolism relevant to calcium absorption by the gastrointestinal tract: (1) hepatic metabolism; (2) renal metabolism; (3) vitamin D transport; and (4) regulation of the renal-vitamin D endocrine system. Certain important areas of vitamin D research, considered less relevant to the topic of calcium absorption, will not be reviewed. These include the actions of vitamin D on bone and kidney function and the possible roles of metabolites other than 1,25-dihydroxyvitamin D_3.

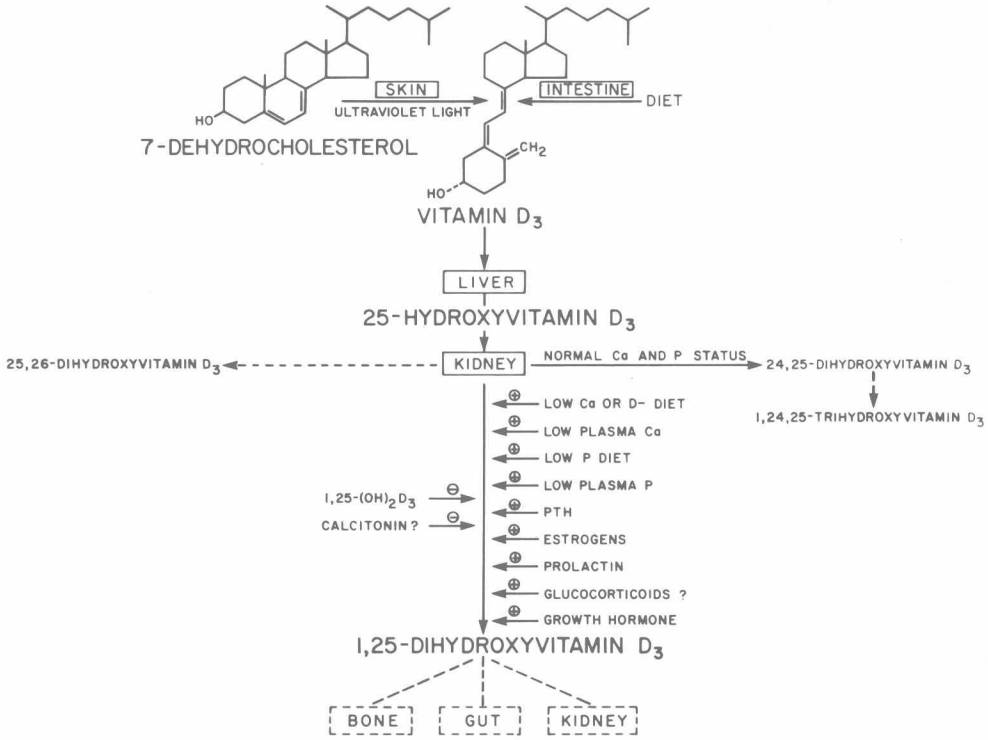

FIGURE 1. Overall scheme of the major metabolic pathways of vitamin D metabolism indicating possible physiological, nutritional, and pharmacological regulatory factors. Vitamin D_3 is synthesized photochemically in the skin from its precursor, 7-dehydrocholesterol. It is transported in the blood bound to vitamin D binding protein and is converted by the liver to 25-hydroxyvitamin D_3 by a hydroxylation reaction which is subject to minimal regulation. The liver metabolite, 25-hydroxyvitamin D_3, circulates at around 30 ng/mℓ, most of which is firmly bound to vitamin D binding protein. The kidney converts 25-hydroxyvitamin D_3 either to 1,25-dihydroxyvitamin D_3, the active hormonal form of vitamin D_3, or to less active forms such as 24,25-dihydroxyvitamin D_3. Tight regulatory control is exercised over the renal 25-hydroxyvitamin D_3-1-hydroxylase enzyme. Of the regulatory factors listed, parathyroid hormone is considered to play a major physiological role. Ingested vitamin D_3 and vitamin D_2 undergo similar pharmacokinetic behavior. Not indicated are the water-soluble, side-chain oxidation products of 1,25-dihydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3, namely calcitroic acid and cholacalcioic acid, respectively (Table 1, Chapter 10).

Chapter 1

HEPATIC METABOLISM OF VITAMIN D

I. NORMAL METABOLISM

Although Kodicek was the first to study the metabolic fate of radioactively labeled vitamin D, he was unable to report the production of any biologically active metabolites. The availability of labeled vitamin D_3 of high specific activity enabled Lund and DeLuca[8] to demonstrate the existence in rats of a metabolite which was active with respect to curing rickets in rats. This metabolite was subsequently isolated in pure form from pig[2] and human[9] plasma and identified as 25-hydroxyvitamin D_3. The liver was determined to be the major production site of this metabolite.[3,10,11] There have been reports of extrahepatic sites in the rat but these sites are claimed to be of minor significance.[3,10,12] These extrahepatic sites, particularly in the intestine and kidney, appear to be more significant in the chicken.[13–15]

A large proportion of administered vitamin D rapidly accumulates in the liver of rats.[16–19] Within the liver, a vitamin D_3-25-hydroxylase enzyme responsible for converting vitamin D to 25-hydroxyvitamin D is located in the smooth endoplasmic reticulum (microsomal) fraction both in rats[20,21] and in chickens.[15] The microsomal enzyme of rat liver is supported by NADPH, molecular oxygen, and a cytoplasmic protein, and is classified as a mono-oxygenase or mixed-function oxidase.[22] Controversy exists over whether or not the microsomal enzyme is cytochrome P-450 dependent and inducible by phenobarbital. Devlin et al.[23] maintain it is, whereas Madhok et al.[22] claim it is not. Another vitamin D_3-25-hydroxylase has been reported to be present in the mitochondria of rat livers.[21] This enzyme also requires NADPH and molecular oxygen and is classified as a mixed-function oxygenase. It is inhibited by carbon monoxide, requires cytochrome P-450, and is subject to induction by phenobarbital.[21] The relative contributions of these two enzymes to the 25-hydroxylation of vitamin D is unclear.

It is well known that vitamin D_2 (ergocalciferol) is approximately ten times less active in the chicken than vitamin D_3 (cholecalciferol).[24,25] No definitive explanation for this discrimination has been proposed. Jones et al.[26] examined the hepatic conversion of vitamin D_2 to 25-hydroxyvitamin D_2 using liver homogenates prepared from immature rachitic chickens, and compared it with the conversion of vitamin D_3 to its 25-hydroxylated metabolite. No difference in the hydroxylation reactions were observed and they concluded that the basis for the discrimination must be elsewhere.

II. REGULATION OF HEPATIC 25-HYDROXYLASE

Although regulation of hepatic 25-hydroxylase activity has been proposed to be present in both rats[27] and chickens,[14] the weight of the evidence indicates that the enzyme is not tightly controlled, at least under *chronic* conditions. Bhattacharyya and DeLuca[14,27] found that administration of *acute* doses of vitamin D_3 decreased hepatic 25-hydroxylase activity; circulating levels of the product of the enzyme, 25-hydroxyvitamin D_3, were not implicated as exerting the regulatory influence. This claim that 25-hydroxylase activity is regulated by *acute* administration of vitamin D is supported by acute studies using the perfused rat liver preparation.[28,29] There is agreement that the mechanism of this acute regulation is not through product

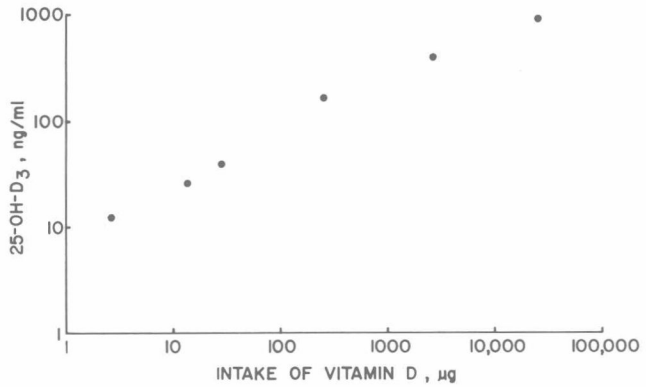

FIGURE 1. Serum 25-hydroxyvitamin D_3 levels in vitamin D-deficient rats supplemented with increasing doses of vitamin D_3. The data are presented in a logarithmic mode on both axes. There is a linear relationship between the intake of vitamin D_3 and the serum level of 25-hydroxyvitamin D_3. (From Clarke, M. B., and Potts, J. T., Jr., *Calcif. Tissue Res.*, 22S, 29, 1977. With permission.)

inhibition of the 25-hydroxylase enzyme,[13,16,23,27,29] as had been proposed originally by Horsting and DeLuca.[30] Other studies conclude that little regulation of hepatic 25-hydroxylase activity is exerted *chronically* either in rats[31] or in man.[32,33] The data obtained by Clark and Potts[31] in rats are presented in Figure 1. In this study young rats were first made vitamin D-deficient and then placed on diets containing different amounts of vitamin D ranging from 0.032 µg to 2.5 mg once or three times weekly. Plasma 25-hydroxyvitamin D levels were determined at weekly intervals and showed no evidence of reaching a plateau.

The explanation for this apparent confusion — whether or not hepatic 25-hydroxylase activity is regulated under physiological conditions — may reside in an examination of the kinetics of the enzyme. In an important study, Fukushima et al.[28] perfused rat livers removed from vitamin D-deficient rats and found 25-hydroxylation occurred in two modes: (1) a fast, high-affinity (K_m, 5.6 n*M*), low-capacity reaction; and (2) a slow, low-affinity (K_m, 1000 n*M*), high-capacity reaction. It is proposed that the high-affinity mode is responsible for 25-hydroxylation of *physiological* levels of vitamin D_3 (<2.5 n*M*), and that the low-affinity mode comes into play with supraphysiological or toxic levels of the prohormone (<2.5 n*M*). Devlin et al.,[23] using microsomal preparations obtained from vitamin D-deficient and vitamin D-replete rats, found that the K_m of the 25-hydroxylases differed under two nutritional states (D-deficient, K_m = 180 n*M*; D-replete, K_m = 440 n*M*). Although Devlin et al.[23] claim that their data are at variance with those of Suda and his colleagues,[28] the lowest substrate concentration used was 20 n*M*, considerably above the K_m (5.6 n*M*) of the high-affinity mode of the 25-hydroxylation reaction reported in the Japanese study. It is understandable, in the light of these kinetic considerations, that regulation is observed in certain studies depending upon the conditions. Acute injection of vitamin D into vitamin D-deficient animals might rapidly saturate the fast high-affinity/low-capacity mode and move into the slow low-affinity/high-capacity mode. This would have the appearance of decreasing the 25-hydroxylase activity. Most of the chronic studies, in which regulation appears to be absent, may be involved only with the slow low-affinity/high-capacity mode.

Whereas vitamin D_3, the natural substrate for hepatic 25-hydroxylase, is regulated

to some degree, the pharmacologically important synthetic derivatives, 1α-hydroxyvitamin D_3[28] and dihydrotachysterol$_2$,[27] appear to be subject to little or no regulation. Both agents, inactive per se, require only 25-hydroxylation by the liver to render them biologically active.

There has been a preliminary report that estrogens may regulate hepatic 25-hydroxylase activity in Japanese quail.[34] Egg-laying birds had greater hepatic 25-hydroxylase activity than did immature birds or mature males; injection of estrogen enhanced the activity in the latter birds.

III. HEPATIC 25-HYDROXYLASE IN LIVER DISEASE

Bone pathology is frequently associated with liver disease, whether the latter is in patients with primary biliary cirrhosis[35] or with alcoholic cirrhosis.[36] Naturally, attention was turned to the question whether or not hepatic 25-hydroxylation was seriously impaired in these conditions.

In patients with primary biliary cirrhosis, circulating levels of 25-hydroxyvitamin D were found to be low (5 ng/mℓ) when compared with patients with normal liver function (9–44 ng/mℓ).[37,38] In those patients treated with monthly intramuscular injections of 100,000 units of ergocalciferol, serum 25-hydroxyvitamin D levels were normal (24 ng/mℓ), indicating that there was no absolute block in the 25-hydroxylation mechanism. Whether the source of the 25-hydroxyvitamin D was hepatic or extrahepatic is not clear. Alcoholic cirrhosis is also associated with low plasma levels of 25-hydroxyvitamin D.[39–42] Again, the impairment in 25-hydroxylation is not absolute; chronic treatment with vitamin D will raise the plasma levels of 25-hydroxyvitamin D to normal or above normal.[39,41,42] The impairment appears to be more extensive when studied following *acute* administration of vitamin D: no significant rise in serum 25-hydroxyvitamin D concentration was seen 24 hr after intravenous administration of 120 μg (4800 units) of cholecalciferol.[40]

Children with hepatitis and congenital biliary atresia have likewise been observed to have low plasma 25-hydroxyvitamin D levels (5.7 and 2.2 ng/mℓ, respectively) when compared with those found in normal children (19.5 ng/mℓ.[33] A marked difference in response to oral administration of 2000 units per day of ergocalciferol for 2 weeks was observed between the hepatitis and the congenital biliary atresia groups. The former responded by an elevation of their plasma 25-hydroxyvitamin D levels (from 8.0 to 22.1 ng/mℓ), whereas no significant response was seen in the children with congenital biliary atresia.

IV. HEPATIC 25-HYDROXYLASE IN ANTICONVULSANT THERAPY

In 1970, reports began to appear that individuals on long-term anticonvulsant therapy such as phenobarbital and phenytoin (Dilantin®) often developed bone disease resembling that seen in vitamin D deficiency.[43–45] Early data indicated that plasma levels of 25-hydroxyvitamin D were low in these patients,[44,46] and the effects of anticonvulsants were attributed to accelerated degradation of vitamin D and 25-hydroxyvitamin D to more polar inactive metabolites by the liver.[44] It was tacitly assumed that the lower levels of 25-hydroxyvitamin D would naturally lead to depressed levels of the physiologically active form of vitamin D, 1,25-hydroxyvitamin D. However, the more recent report that in patients on chronic anticonvulsant therapy plasma 1,25-hydroxyvitamin D levels were not depressed, but were either normal or elevated,[47] did not confirm this assumption. These studies were supported