3

VITAMIN D METABOLISM

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1. INTRODUCTION

The demonstration that 1,25-(OH)$\textsubscript{2}$D$_3$ is a hormone which governs calcium homeostasis has focused attention on the mechanisms of 1,25-(OH)$\textsubscript{2}$D$_3$ action, and the processes that regulate its production. Much less attention has been directed to the other metabolites of 25-OH-D$_3$, and most of that has concerned 24R,25-(OH)$\textsubscript{2}$D$_3$, a compound whose function is controversial. The complex metabolism of 25-OH-D$_3$, other than 1$\alpha$-hydroxylation, may be a degradative mechanism. It is equally conceivable that there are other functions of vitamin D, and that metabolites of 25-OH-D$_3$ and 1,25-(OH)$\textsubscript{2}$D$_3$ support such functions in bone, kidney, intestine, and muscle. It is the purpose of this review to concentrate on developments in vitamin D metabolism that have occurred since 1978, which might affect appraisal of the importance of 25-OH-D$_3$ metabolism, other than through 1$\alpha$-hydroxylation. Advances in the methods used to study vitamin D metabolism, especially high performance liquid chromatography (HPLC) and mass spectroscopy, will be discussed. The reader is directed to a number of reviews for a broad view of vitamin D metabolism and action (1-7).

2. METHODS USED IN THE PURIFICATION AND IDENTIFICATION OF VITAMIN D METABOLITES

Sephadex LH-20 chromatography is very useful in purification of vitamin D metabolites prior to HPLC. But several of the known vitamin D metabolites co-migrate on Sephadex LH-20 (8). It is likely that uncharacterized metabolites also co-migrate with known derivatives. Consequently, co-migration of standards and unknowns on Sephadex LH-20 is a poor technique for ascertaining co-specificity. Furthermore, pre-separation of substrate and products from extracts with Sephadex LH-20 may give incomplete product recovery as a result of products migrating.
with substrate or with known metabolites. Analysis of only the new peaks, or of only the peaks of interest, may provide incomplete information concerning the metabolic conversions of substrate.

Figure 1. Migration of peak Z and 25-OH-D₃-lactone in three chemically different analytical HPLC systems: A) silica gel developed with a gradient of 1 to 14% 2-propanol in hexane; B) silica gel developed with hexane/methanol/chloroform (22/1/2); C) amino derivatized silica gel (Zorbax-NH₂) developed with 2-propanol/hexane (6/94).

HPLC has emerged as the method of choice for the rigorous analysis of vitamin D metabolites. HPLC, however, is not a fool-proof technique. As described in the chapter by R. L. Horst, several metabolites of vitamin D₃ and vitamin D₂ co-migrate on silica gel HPLC columns developed with hexane/isopropanol. These metabolites can be separated by further chromatography on silica gel HPLC columns developed with methylene chloride/isopropanol. But sometimes even this is not sufficient. For example, in studies of 23,25-(OH)₂D₃ metabolism by chick kidney homogenates, the then unknown compound, 23-keto-25-OH-D₃ was isolated (9-11). Incubation of 23-keto-25-OH-D₃ with chick kidney homogenates produced a metabolite (peak Z) that comigrated with 25-hydroxyvitamin D₃-26,23-lactone (25-OH-D₃-lactone) on an analytical silica gel HPLC column developed in a hexane/isopropanol gradient system (Fig. 1A). When peak Z was rechromatographed on an analytical silica gel HPLC column, developed in a hexane/methanol/chloroform system, the compound co-migrated a second time with 25-OH-D₃-lactone (Fig. 1B). The evidence, therefore, suggested that peak Z was 25-OH-D₃-lactone. This was not the case, as shown by analysis of peak Z on an analytical amino HPLC column (Fig. 1C).