This paper described a specific assay for the quantitation of the major circulating metabolite of vitamin D in plasma. Following ether extraction of plasma and stepwise chromatography in Pasteur pipettes, radioinert 25-OH$_3$D$_3$ competes with 25-OH[3H]D$_3$ for binding to the plasma vitamin D binding protein. The technique permitted facile measurements of a metabolite constitutively produced from vitamin D, thereby allowing indirect studies of endogenous vitamin D biosynthesis and nutriture, as well as the pharmacological applications of vitamin D. [The SC® indicates that this paper has been cited in more than 725 publications.]

**Specific Plasma Vitamin D Metabolite Measurements**

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In 1968, I had the good fortune to enter a stimulating endocrinology fellowship program at the Washington University School of Medicine and come under the influence of a remarkably energetic and supportive mentor, Louis V. Avioli, at the Jewish Hospital of St. Louis. Although the intermediary metabolism of vitamin D was just becoming understood, the potent, hormonal form of the vitamin [1,25-(OH)$_2$D] had yet to be identified, and assays then available were the tedious bioassay and multistep chromatography followed by spectroscopy. For tracking conversions of $^3$H-cholecalciferol carried out in vivo, we applied tissue extracts to multibore columns of silicic acid and carried out five hyperbolic, multisolvant elution steps in a multigridded corner of the laboratory, best visualized as a glassblower’s dream come true. When not in the lab, my clinical time often involved interpreting hormone levels that were increasingly being analyzed by sterospecific assays that utilized natural binding proteins.

During the period of transition to junior faculty status, I became obsessed with the nature of the plasma transport of the major metabolite, 25-OH$_3$. With the crucial help of my first technician, Kyung Ja Chyu, we studied the human plasma binding of 25-OH$_3$, and this led to our efforts to develop a competitive radioassay for this sterol. We abbreviated the chromatography and used a polar solvent extraction in order to select 25-OH$_3$ and avoid unpolar lipids. 25-OH[3H]D$_3$ was just becoming available commercially, and serum or a rat kidney extract provided the selective, high-affinity binding protein. The techniques were relatively simple and the demand to measure the major circulating metabolite of vitamin D was certainly apparent from the flow of scientists who came to the lab to learn the assay directly. This was gratifying, as was the ability to enter into collaborative projects with other investigators.

Over the last 20 years, the assay technology for vitamin D and its metabolites has developed well, and current clinical discussions routinely include data from vitamin D metabolite assays carried out by commercial laboratories. Although my lab no longer assays 25-OH$_3$ routinely, my major research focus continues to be the remarkably multifunctional plasma binding protein for vitamin D and its metabolites (DBP, Gc globulin). I have fond memories of the 25-OH$_3$ assay development years, and I’m pleased that the assay (or its modifications) has been helpful to many investigators.