

This Week's Citation Classic

Fraser D R & Kodicek E. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature* 228:764-6, 1970.

[Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council, Cambridge, England]

A vitamin D 1-oxygenating enzyme was discovered in chicken kidney mitochondria. This produces the metabolite now known to be 1,25-dihydroxyvitamin D(1,25(OH)₂D). In rats it was shown that the 1-hydroxylase was present only in kidney thus revealing a new endocrine function for this organ in controlling calcium metabolism. [The SC¹® indicates that this paper has been cited in over 785 publications since 1970.]

David R. Fraser
Dunn Nutritional Laboratory
University of Cambridge
and
Medical Research Council
Cambridge CB4 1XJ
England

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"In the 1960s, studies on the mechanism of action of vitamin D were testing the hypothesis that it functioned as a steroid hormone in cell nuclei and induced specific proteins involved in calcium transport. With the discovery by DeLuca's group in Wisconsin¹ of the biologically active metabolite, 25-hydroxyvitamin D, attempts were made to locate this substance in the nuclei of intestinal cells. Eric Lawson in Cambridge then found that there was a derivative of 25-hydroxyvitamin D in these nuclei: a metabolite characterised by its serendipitous loss of tritium from the 1-alpha position of tritium-labelled vitamin D.²

"As my own research was floundering at that time, the director of the Dunn, Egon Kodicek, suggested that I try to find an enzyme which produced this new metabolite. The enzyme could then be used to generate the metabolite *in vitro* in amounts required to complete its chemical identification. It was assumed first that the enzyme might be a steroid hydroxylating mixed-function oxidase and secondly that its location was probably at the site of highest metabolite concentration—the intestinal mucosa. The first assumption

eventually proved to be correct but the second was dispiritingly wrong. After several futile months incubating labelled 25-hydroxyvitamin D with all manner of intestinal preparations, the search was diverted to other tissues. By a combination of homogenate incubations and surgical extirpations, a wide range of organs was found not to contain the enzyme. The most unpredicted and only remaining site, the kidney, was finally shown on October 14, 1969 to make the metabolite *in vitro*. Because this was the one positive finding, nephrectomized rats were then used to confirm that the enzyme was functioning only in the kidney *in vivo*.

"For the next 12 months, I had the delightful and luxurious privilege of studying a fascinating new renal enzyme system of which the world outside the Dunn was still unaware. This research was finally reported in *Nature* in November 1970. Subsequently, medical and endocrine use of 1,25(OH)₂D made the study of its formation in the kidney a very popular topic. For a recent review, see *Physiological Reviews*.³ Despite its popularity, this *Nature* paper is not of special importance. It describes just one step in a sequence of research in several laboratories which revealed the functional metabolism of vitamin D. The discovery of the 1-hydroxylase was notable for a complete absence of intellectual wisdom—a search for a needle in a haystack by the tedious inspection of each straw. A number of other research groups would, with time, have stumbled on the same result. This particular search was successful because of a simple assay for a tritium-deficient product on thin-layer chromatography and a realisation that only µg amounts of 1,25(OH)₂D would be formed *in vitro*.

"This paper may be widely cited, partly because all the work was published only once and partly because there have now been large numbers of papers on vitamin D which all needed a convenient reference to the renal 1-hydroxylase. Regardless of any historical significance, this research was great fun to do, giving a lot of pleasure to myself and much satisfaction to Kodicek who sadly died in July 1982."

1. Blunt J W, DeLuca H F & Schnoes H K. 25-Hydroxycholecalciferol. A biologically active metabolite of vitamin D₃. *Biochemistry* 7:3317-22, 1968.
2. Lawsoa D E M, Wilson P W & Kodicek E. New vitamin D metabolite localized in intestinal cell nuclei. *Nature* 222:171-2, 1969.
3. Fraser D R. Regulation of the metabolism of vitamin D. *Physiol. Rev.* 60:551-613, 1980.