This Week's Citation Classic®

Wasserman R H & Taylor A N. Vitamin D_3-induced calcium-binding protein in chick intestinal mucosa. *Science* 152:791-3, 1966.
[Dept. Physical Biology, New York State Veterinary Coll., Cornell Univ., Ithaca, NY]

This paper provided the first conclusive evidence for the existence of a specific calcium-binding protein that was synthesized in response to vitamin D administration. Additionally, the temporal appearance of the protein in intestinal tissue suggested an involvement in the vitamin D-mediated calcium-absorption process. Subsequent studies over the years have documented the presence of the protein in other tissues and organs where it is now regarded as an end-organ marker of the vitamin D endocrine system. [The *SCI*® indicates that this paper has been cited in over 460 publications.]

Robert H. Wasserman
Department of Physiology
Cornell University
Ithaca, NY 14853-6401
and
Alan N. Taylor
Department of Anatomy
Baylor College of Dentistry
Dallas, TX 75246

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The studies reported in this *Citation Classic* support the concept that there is indeed such a thing as serendipity, i.e., a fortuitous discovery that was not the original target of the study. The experiments that led up to the cited publication took place in the early 1960s, at a time when our knowledge of the mechanism of vitamin D action was very primitive compared to today.

Prompted by reports indicating that vitamin D enhanced uphill intestinal calcium transport[1] and release of calcium by kidney mitochondria,[2] we attempted to quantitate the compartmentalization of calcium in the enterocyte during the absorption process. Differential centrifugation was used to separate the subcellular fractions of homogenates of intestinal mucosa after varying periods of *in vivo* absorption of radiocalcium. It was immediately apparent that prior vitamin D administration had a pronounced effect on radiocalcium distribution in the subcellular fractions, with the soluble fraction from vitamin D-treated chicks having severalfold more calcium than the rachitic controls. Some unexplained negative results from ultracentrifugation studies delayed for a time the ultimate realization that a vitamin D-induced calcium-binding protein in the soluble phase was responsible for the observed results. The trypsin digestion experiments reported in the *Citation Classic*, plus electrophoretic separation and visualization, provided decisive evidence for the existence of the unique protein.

Significant progress towards understanding the molecular basis of vitamin D action was made in 1959 with the report that vitamin D increased uphill calcium transport by the intestine.[1] Shortly thereafter, in the mid- to late 1960s, studies on the metabolism of vitamin D revealed the formation of metabolites more biologically active than vitamin D in the liver (25-OH-D_3)[3] and the kidney (1,25-$(OH)_2$-D_3).[4] A genomic action of vitamin D_3 was proposed when inhibitors of protein synthesis were shown to depress vitamin D-dependent calcium absorption,[5] and our studies reported in the *Citation Classic* gave the first evidence of the existence of a unique calcium-binding protein inducible by vitamin D, thus providing direct support of the genomic theory of vitamin D action.

Because the vitamin D-induced protein bound Ca^{2+} with high affinity, an immediate connection could be made between the physiological action of vitamin D and this protein. In fact, the correlation coefficient between the concentration of this protein in the intestine and the efficiency of calcium absorption in a variety of studies was 0.9 or better.[6] An analogous vitamin D-induced calcium-binding protein of lower molecular weight was identified in mammalian intestine,[7] and these proteins are now named "calbindin-D_{28k}" and "calbindin-D_{9k}" for the avian-type and intestinal mammalian-type, respectively (the subscripts refer to the approximate molecular weights in kilodaltons). The calcium-binding properties, amino acid composition, and amino acid sequences[8] of these proteins have been determined, and the three-dimensional structure of bovine intestinal calbindin-D_{9k} was recently reported.[9]

In the chick, calbindin-D_{28k} is present in the intestine, kidney, shell gland, pancreas, brain, peripheral nerve, and other tissues. In mammalian species, calbindin-D_{9k} is found primarily in the intestine, and a calbindin-D_{28k} similar but not identical to that of the avian species is present in the kidney, placenta, pancreas, brain, and other tissues. Thus, the localization studies showed that the protein was not relegated exclusively to epithelial-type tissues as originally thought, but was found in nonepithelial-type tissues as well. The function of the protein has not been absolutely clarified, although it is considered to increase the translocation of Ca^{2+} through the cytosol of the enterocyte, and this mechanism could pertain to other epithelial tissues. In nonepithelial cells the protein might serve essentially as a calcium buffer, modulating the concentration of intracellular calcium, particularly upon stimulation by specific agonists.

1. **Schachter D & Rosen S M.** Active transport of Ca^{45} by the small intestine and its dependence on vitamin D. *Amer. J. Physiol.* 196:357-62, 1959. (Cited 355 times.)
2. **Engstrom G W & DeLuca H F.** The action of vitamin D *in vivo* and *in vitro* on the release of calcium from kidney mitochondria. *J. Biol. Chem.* 237:PC974-5, 1962. (Cited 55 times.)
3. **Lund J & DeLuca H F.** Biologically active metabolite of vitamin D_3 from bone, liver, and blood serum. *J. Lipid Res.* 7:739-44, 1966. (Cited 270 times.)
4. **Cousins R J, DeLuca H F, Suda T, Chen T & Tanaka Y.** Metabolism and subcellular location of 25-hydroxycholecalciferol in intestinal mucosa. *Biochemistry* 9:1453-9, 1970. (Cited 75 times.)
5. **Zull J E, Czarnowska-Misztal E & DeLuca H F.** Actinomycin D inhibition of vitamin D action. *Science* 149:182-4, 1965. (Cited 120 times.)
6. **Morrissey R L & Wasserman R H.** Calcium absorption and calcium-binding protein in chicks on differing calcium and phosphorus intakes. *Amer. J. Physiol.* 220:1509-15, 1971. (Cited 175 times.)
7. **Kallfelz F A, Taylor A N & Wasserman R H.** Vitamin D-induced calcium binding factor in rat intestinal mucosa. *Proc. Soc. Exp. Biol. Med.* 125:54-8, 1967. (Cited 105 times.)
8. **Fullmer C S & Wasserman R H.** Chicken intestinal 28-kilodalton calbindin-D: complete amino acid sequence and structural considerations. *Proc. Nat. Acad. Sci. USA* 84:4772-6, 1987.
9. **Szebenyi D M E, Obendorf S K & Moffat K.** Structure of vitamin D-dependent calcium-binding protein from bovine intestine. *Nature* 294:327-32, 1981. (Cited 110 times.)