The Discovery of Vitamin D: The Contribution of Adolf Windaus

George Wolf[1]

Department of Nutritional Sciences and Toxicology, University of California, Berkeley, CA 94720-3104

The Nobel prize for chemistry for 1928 was awarded to Adolf Windaus "for his studies on the constitution of the sterols and their connection with vitamins" (1), the first person to receive an award mentioning vitamins. What was the contribution Windaus made to our knowledge of vitamins that deserved the highest scientific accolade?

The vitamin in question was vitamin D. It had a long history before Windaus appeared on the scene. Rickets, the bone disease caused by vitamin D deficiency, was known in antiquity and was described in detail by F. Glisson in 1650 (2). Many causes and cures for rickets had been proposed. Although cod-liver oil had been used medicinally for a long time, D. Scheutte (2) in 1824 was the first to prescribe it for the treatment of rickets. It was not until 1906 that Hopkins (3) postulated the existence of essential dietary factors necessary for the prevention of diseases such as scurvy or rickets.

The first scientific approach to the disease was made by McCollum and his co-workers. In their early research (4) in 1914, they isolated a fat-soluble, nonsaponifiable factor from butterfat, necessary for normal growth and prevention of the eye disease xerophthalmia in young rats. They named this factor "fat-soluble factor A," later "vitamin A." The notion of a fat-soluble essential dietary factor for health led Mellanby (5) in 1919 to experiment with puppies in which he succeeded in producing a bone disease by feeding them a diet of low-fat milk and bread. He diagnosed rickets by X-ray examination, bone-calcium assay, and histology of bone, and noted that the gross appearance of the dogs was quite similar to that of rachitic children. Even adding yeast to the dogs' diet (to provide the water-soluble B-vitamins) and orange juice (to prevent scurvy), did not prevent the appearance of rickets within 3–4 mo. Rickets was prevented by the addition of butterfat to their diet or, most effectively, of cod-liver oil. He wrote: "Rickets is a deficiency disease which develops in consequence of the absence of some accessory food factor or factors. It therefore seems probable that the cause of rickets is a diminished intake of an anti-rachitic factor, which is either [McCollum's] fat-soluble factor A, or has a similar distribution to it" (5). A landmark investigation was that of Hariette Chick and her co-workers (6) who, in 1922, working with malnourished children in a clinic in post-World War I Vienna, showed that rickets prevalent in the children could be cured by whole milk or cod-liver oil.

In 1920 Hopkins (7) found that the fat-soluble factor A in butterfat could be destroyed by heating and aeration. Butterfat so treated no longer had growth-promoting activity; the rats fed the treated butterfat developed xerophthalmia and died within 40–50 d.

The key experiment was performed by McCollum and his co-workers (8) in 1922, when they observed that heated, oxidized cod-liver oil could not prevent xerophthalmia but could cure rickets in the rats. "This shows that oxidation destroys fat-soluble A without destroying another substance which plays an important role in bone growth" (8). They concluded that fat-soluble factor A consisted of 2 entities, one later called "vitamin A," the other being the newly discovered antirickets factor. Because the water-soluble factors then discovered were termed vitamin B and the known antiscurvy factor was called vitamin C, they named the new factor vitamin D.

In the meantime, an entirely different cure for rickets appeared, in the role of UV light. A long-standing tradition held that fresh air and sunshine were good for the prevention of rickets. Hess and Unger (9), in 1921, put forward the explanation of their clinical observations that "seasonal incidence of rickets is due to seasonal variations of sunlight." In her work with children, Chick and her team (6), mentioned above, observed that sunlight would cure rickets just as well as cod-liver oil.

The field received a new impetus when Huldschinsky (10) in 1919 argued that, if sunlight at the seaside or in the mountains can prevent or cure rickets, then artificial sunlight, simulating light at mountain heights ("Höhensonne") should do the same. He exposed severely rachitic children to irradiation with a quartz-mercury lamp (emitting UV light) every other day for 2 to 20 min for 2 mo and observed great improvement, including fresh calcium deposition, as revealed by X-rays. He was careful to make sure that the children had not been exposed to sunlight or received any supplements to their diet during those months.

The thinking at that point was that rickets can be prevented or cured by a component of butterfat or cod-liver oil that was distinct from the fat-soluble factor A (vitamin A). It can also be cured by an entirely different process, by sunlight or UV light irradiation simulating sunlight, perhaps as the result of generally improved health.

This dichotomy was jolted by the most surprising observations, made simultaneously in 1924 in 3 different laboratories. Hume and Smith (11,12) found that rats suffering from rickets induced by a low-phosphate diet (13) benefited from irradiation by UV light, not only by irradiation of the rats themselves, but also by irradiation of the "air" in the glass jars from which they had been removed and then put back after irradiation. It turned out that it was the irradiated sawdust, feces,

[1] To whom correspondence should be addressed.
E-mail: retinol@nature.berkeley.edu.

0022-3166/04 $8.00 J. Nutr. 134: 1299–1302, 2004.
Manuscript received 12 January 2004.

and spilt food left in the jars, which the rats later ate, and not the air that improved the rats' rickets.

Goldblatt and Soames (14) observed that livers of irradiated rats were curative when fed to rachitic rats. Steenbock and Black (15) went a step further. They argued that because liver in the *living* rat is activated by UV light, perhaps liver *removed* from the animals can also be so activated. Indeed, both liver and muscle tissue from nonirradiated rats, after removal from the body and exposed to UV light "was found to have become activated, being both growth-promoting and bone-calcifying," when fed to nonirradiated rachitic rats.

The third laboratory that simultaneously reported the imparting of antirachitic properties to inert foods, such as wheat, lettuce, or cottonseed oil, was that of Hess and Weinstock (16,17). They also showed that in linseed oil, the antirachitic properties resided in the nonsaponifiable fraction and that activation occurred by UV irradiation in the absence of oxygen (18).

The discovery that irradiation of food, in particular of whole milk (containing butterfat), could impart antirachitic potency led to tremendous advances in public health. The procedure, when adopted by producers, caused a rapid decline in the prevalence of rickets in children.

It was well known at that time (1924) that certain animal fats, such as butterfat or cod-liver oil, were antirachitic without irradiation. What, then, was the substance in vegetable oils that could be activated by irradiation? In 1925, Hess and his team (19) isolated sitosterol (then called phytosterol) from cottonseed oil, an abundant fraction in the nonsaponifiable portion of the oil. What appeared to the investigators to be sitosterol was inactive against rickets in rats. Upon irradiation by UV light, it became active. Similarly, cholesterol, isolated from rat brain and recrystallized to a state thought by the authors to be pure, was activated to become an antirachitic substance by irradiation. Therefore, at this time (1925), the conclusion was reached that the precursor of the active substance, susceptible to activation by UV light, was cholesterol.

With amazing foresight, Hess et al. (19) proposed the hypothesis that "it would seem quite possible that the cholesterol [we now know that this is 7-dehydrocholesterol] in the skin is normally activated by UV-irradiation and rendered anti-rachitic—that the solar rays and artificial radiations can bring about this conversion. This point of view regards the superficial skin as an organ, which reacts to particular light waves rather than as a mere protective covering."

At this moment (1926) some doubts arose as to the purity of the "pure" cholesterol, convertible into the antirachitic substance. Heilbron et al. (20) had observed that the "pure" cholesterol samples showed spectroscopic absorption peaks in the UV region that could not have belonged to cholesterol. Cholesterol was known to have a single double bond and the 3 peaks found by Heilbron et al. (20) would have been due to 2 or 3 double bonds. The suspicion arose that "pure" cholesterol, as obtained from rat brain, contained a small amount of an impurity that may be the precursor of the vitamin.

It was at this stage (in 1926) that A. F. Hess (in New York) asked the famous steroid chemist A. Windaus (in Göttingen, Germany) to collaborate on the question of the chemical structure of the antirachitic product formed by irradiation of the substance then thought to be cholesterol (21). A third investigator, who took part in this exceptionally amicable collaboration, was O. Rosenheim in London.

Because physical methods, such as recrystallization, left the supposedly pure cholesterol unchanged, chemical methods were tried. Thus, Rosenheim and Webster, at a meeting of the Biochemical Society in London in 1926 (22) announced that "the precursor of vitamin D is not cholesterol itself, but a substance which is associated with and follows 'chemically pure' cholesterol in all its stages of purification by the usual methods (saponification and recrystallization)." The investigators converted the "pure" cholesterol into its dibromide, recrystallized this, and recovered cholesterol upon treatment with sodium amalgam. When purified by this method, the recovered cholesterol, upon irradiation, had completely lost its antirachitic potency. The authors state (22): "According to information received from Prof. Windaus, a specimen of cholesterol [was] prepared by him *at our suggestion* [my italics] by the same procedure. Thus purified, cholesterol prepared in this way was no longer rendered anti-rachitic by irradiation with ultraviolet light. It is evident, therefore, that not cholesterol, but [another] substance is the immediate precursor of vitamin D."

From this report, it is clear that, in the close collaboration among Rosenheim, Hess, and Windaus, it was Rosenheim's team that performed the crucial experiment demonstrating that "pure" cholesterol contained an impurity that could be converted into the antirachitic vitamin photochemically. This was the essential clue that led to the identification of the vitamin D precursor or provitamin.

Windaus and Hess (21) improved upon the chemical purification of the provitamin by the debromination of the cholesterol dibromide by zinc dust. Rosenheim and Webster (23) emphasized that the work resulted from mutual suggestions emanating from the 3 teams in London, Göttingen, and New York simultaneously, "according to a friendly arrangement."

With the discovery that "pure" cholesterol contained a small amount of an impurity that appeared to be the provitamin, the collaborative research began by the 3 groups on its identification. The impurity had the chemical properties of a steroid, being precipitable by digitonin and displayed a spectrum characteristic of the presence of 3 double bonds. Rosenheim and Webster (23) pointed out that the amount of the impurity must be very small (1:2000) and hence the vitamin itself must be biologically active in very small amounts.

The work of the identification of the provitamin was greatly speeded up by the discovery of Heilbron et al. (20), already referred to, that the active impurity had 3 absorption peaks in the UV spectrum (269, 280, 293 nm). Thus, it was possible to purify the provitamin using the UV absorption peaks as a guide instead of the laborious animal tests. Windaus and Hess (21), by means of high-vacuum distillation and charcoal adsorption techniques, obtained the highly concentrated active fraction from "pure" cholesterol.

Windaus and Hess (21) tested 30 different steroid preparations with more than 1 double bond from various plant sources for antirachitic activity upon irradiation. They hit upon ergosterol, a fungal steroid from ergot (**Fig. 1**), which when irradiated, was found to be highly active in curing rats suffering from rickets. The reasons for choosing this steroid for testing were as follows: *1*) its UV spectrum matched that of the active fraction from "pure" cholesterol; *2*) it was a steroid rapidly destroyed by oxidation, similar to the active fraction from cholesterol; *3*) it produced the same color reaction with sulfuric acid as that fraction. Simultaneously and in consultation with Windaus and with Hess, Rosenheim and Webster (23) also determined that ergosterol was the provitamin D, convertible to vitamin D by UV irradiation.

The irradiation product of ergosterol was purified and crystallized simultaneously in 1931 by the London team (24), by Reerink et al. (25) in the Netherlands, and by Windaus and his co-workers (26). It was named vitamin D-2 or calciferol and showed enormous potency, i.e., 0.01 μg/d given to rachitic

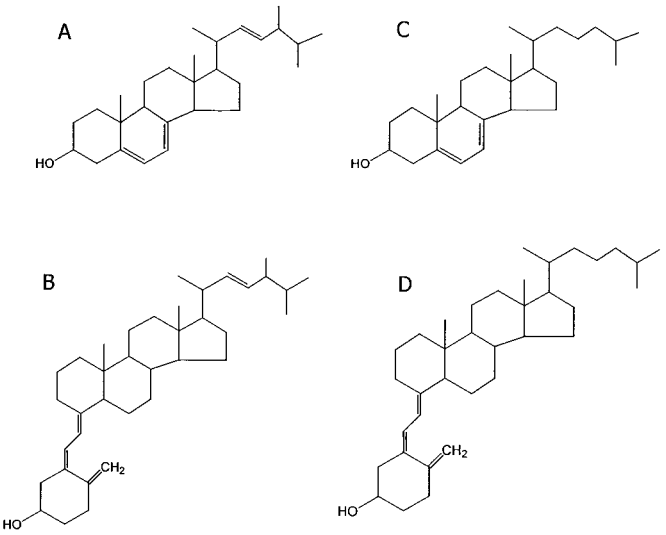

FIGURE 1 Structure of ergosterol (*A*), calciferol or vitamin D-2 (*B*), 7-dehydrocholesterol (*C*), and cholecalciferol or vitamin D-3 (*D*).

rats for 2 mo effected a complete cure. Thanks to the extensive research on steroids by Windaus and his group, they were able to determine the chemical properties of calciferol, i.e., it was isomeric with ergosterol, and it had a hydroxyl group and 3 conjugated double bonds (26). Its correct structure was established by Windaus and Thiele in 1936 (27) (Fig. 1).

The following question remained: because ergosterol does not occur in the animal organism, how can the animal (or human) obtain its vitamin D by sunlight? This important problem was not solved until 1937, long after the Nobel award, when Windaus and Bock (28) isolated and identified a compound, 7-dehydrocholesterol (Fig. 1), from hog skin, also shown to be present in rat and human skin, and in food from animal sources such as whole milk or liver, convertible to an antirachitic substance by irradiation. 7-Dehydrocholesterol was a known compound, having been synthesized from cholesterol by Windaus et al. (29) in 1935. Its irradiation product was named vitamin D-3 (Fig. 1) or cholecalciferol. Its structure was established by Windaus et al. (30), who investigated the complex photochemical reactions in its formation. The complete photochemical and thermal reaction steps from ergosterol to calciferol were elucidated only in 1955 by Velluz et al. (31). The exact sequence of steps leading to the photoproduction of cholecalciferol in the skin were reported in a comprehensive paper by Holick et al. in 1980 (32).

Many scientists contributed to the long story of the discovery of vitamin D, standing out among them H. Steenbock, A. F. Hess, and O. Rosenheim. A. Windaus, as the foremost steroid chemist of the 1920s and 1930s, made a crucial contribution.

Adolf Windaus (1876–1959) studied medicine in Berlin, then switched to chemistry at the University of Freiburg, Germany, under H. Kiliani. Before his work on vitamin D and the award of the Nobel prize in 1928, Windaus devoted his career exclusively to the elucidation of the structure of cholesterol. The title of his inaugural dissertation (1903) was entitled simply "Über das Cholesterin" ("About Cholesterol"). He continued to work on this topic for almost 30 years, until the problem was solved. He was the first to establish the empirical formula for cholesterol and showed it to be a 4-ring compound with a hydroxyl group in 1 ring, a double bond in the β-γ position in an adjoining ring and an 8-carbon side chain. In collaboration with H. Wieland (33), he established the relation of cholesterol to the bile acids, a hitherto unsuspected link between 2 biological substances. However, the relation he proposed among the 4 rings of cholesterol at the time of his Nobel prize (1928) was incorrect. Bernal (34) pointed out that it was incompatible with that deduced from his X-ray crystallographic studies. At the same time, the correct structural skeleton for the closely related bile acids was arrived at chemically by Wieland and Dane (35). The final determination of the now accepted structure of cholesterol was accomplished by X-ray crystallography with cholesteryl iodide by Carlisle and Crowfoot (36) in 1945.

Adolf Windaus stood out among scientists in Germany, in openly opposing the Nazi regime. Butenandt, in his Windaus Memorial Lecture (37) stated: "His sense of justice and love of truth brought Windaus into dangerous opposition to the despots of National Socialism, to whom he would make no concession." As head of the Institute for Organic Chemistry and Professor at the University in Göttingen, he protected a Jewish graduate student from dismissal. He wrote to Butenandt in 1933: "To have to look at injustice and not to be able to help is difficult to bear . . . at this moment people are carrying past placards on which stands the most unbelievable and hellish slander against Jews" (38). His "profound moralistic conscience" (38) caused him to refuse to carry out research on poison gas, even as far back as World War I. Perhaps it was significant that he stopped all scientific research in 1938, at age 62, even though he did not retire until 1944.

With hindsight, one could say that the Nobel prize for chemistry in 1928 should have been shared by Windaus, Rosenheim, and Hess. However, because the citation for the prize for Windaus was "for his studies on the constitution of the sterols and their connection with vitamins" (1), and his contribution to steroid chemistry was immense, of which the chemistry of vitamin D was only a part, he assuredly deserved the prize.

ACKNOWLEDGMENT

I am grateful to Dr. Kenneth J. Carpenter for suggesting to me the topic of this article and for providing some of the basic documentation.

LITERATURE CITED

1. Farber, E. (1953) Nobel Prize Winners in Chemistry. Abelard-Schuman, New York, NY. Windaus' Nobel lecture is available online at http://www.nobel.se/chemistry/laureates/1928/windaus-lecture.html.
2. McCollum, E. V. (1957) A History of Nutrition. Houghton Mifflin Co., Boston, MA.
3. Hopkins, F. G. (1906) The analyst and the medical man. Analyst 31: 385–404.
4. McCollum, E. V. & Davis, M. (1914) Observations on the isolation of the substance in butter fat which exerts a stimulating effect on growth. J. Biol. Chem. 19: 245–250.
5. Mellanby, E. (1919) An experimental investigation on rickets. Lancet 196: 407–412.
6. Chick, H., Dalyell, E.J.H., Hume, E. M., Mackay, H.M.M. & Henderson-Smith, H. (1922) The aetiology of rickets in infants: prophylactic and curative observations at the Vienna University Kinderklinik. Lancet ii: 7–11.
7. Hopkins, F. G. (1920) The effect of heat and aeration upon the fat-soluble vitamine. Biochem. J. 14: 725–733.
8. McCollum, E. V., Simmonds, N., Becker, J. E. & Shipley, P. G. (1922) Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. J. Biol. Chem. 53: 293–312.
9. Hess, A. F. & Unger, L. J. (1921) The cure of infantile rickets by artificial light and by sunlight. Proc. Soc. Exp. Biol. Med. 18: 298.
10. Huldschinsky, K. (1919) Heilung von Rachitis durch künstliche Höhensonne. Dtsch. Med. Wochenschr. 45: 712–713.
11. Hume, E. M. & Smith, H. H. (1924) The effect of irradiation of the environment with ultra-violet light upon the growth and calcification of rats, fed on

a diet deficient in fat-soluble vitamins: the part played by irradiated sawdust. Biochem. J. 18: 1334–1345.
12. Carpenter, J. K. & Zhao, L. (1999) Forgotten mysteries in the early history of vitamin D. J. Nutr. 129: 923–927.
13. McCollum, E. V., Simmonds, N., Shipley, P. G. & Park, E. A. (1921) The production of rickets by diets low in phosphorus and fat-soluble A. J. Biol. Chem. 47: 507–528.
14. Goldblatt, H. & Soames, K. M. (1923) Studies on the fat-soluble growth-promoting factor. Biochem. J. 17: 446–453.
15. Steenbock, H. & Black, A. (1924) Fat-soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultra-violet light. J. Biol. Chem. 61: 405–422.
16. Hess, A. F. & Weinstock, M. (1924) Antirachitic properties imparted to lettuce and to growing wheat by ultraviolet irradiation. Proc. Soc. Exp. Biol. Med. 22: 5.
17. Hess, A. F. & Weinstock, M. (1924) Antirachitic properties imparted to inert fluids by ultraviolet irradiation. Proc. Soc. Exp. Biol. Med. 22: 6–7.
18. Hess, A. F. & Weinstock, M. (1925) A further report on imparting antirachitic properties to inert substances by ultra-violet irradiation. J. Biol. Chem. 63: 297–307.
19. Hess, A. F., Weinstock, M. & Heelman, F. D. (1925) The antirachitic value of irradiated phytosterol and cholesterol. J. Biol. Chem. 63: 305–309.
20. Heilbron, I. M., Kamm, E. D. & Morton, R. A. (1927) The absorption spectrum of cholesterol and its biological significance with reference to vitamin D. Part I. Biochem. J. 21: 78–85.
21. Windaus, A. & Hess, A. (1926) Sterine und antirachitisches Vitamin. Nachrichten von der Gesellschaft der Wissenschaften zu Göttingen, pp. 175–184.
22. Rosenheim, O. & Webster, T. A. (1926) Further observations on the photo-chemical formation of vitamin D. J. Soc. Chem. Ind. 45: 932.
23. Rosenheim, O. & Webster, T. A. (1927) LVI. The parent substance of vitamin D. Biochem. J. 21: 389–397.
24. Askew, F. A., Bruce, H. M., Callow, R. K. Philpot, J. S. & Webster, T. A. (1931) Crystalline vitamin D. Nature (Lond.) 128: 758.
25. Reerink, E. H. & Van Wijk, W. (1931) The vitamin D problem. Biochem. J. 25: 1001–1009.
26. Windaus, A. (1931) The chemistry of irradiated ergosterol. Proc. R. Soc. (Lond.) 108: 568–575.
27. Windaus, A. & Thiele, W. (1936) Über die Konstitution des Vitamins D_2. Ann. Chem. 521: 160–175.
28. Windaus, A. & Bock, F. (1937) Über das provitamin aus dem Sterin der Schweineschwarte. Z. Physiol. Chem. 245: 168–170.
29. Windaus, A., Lettré, H. & Schenck, F. (1935) Über das 7-Dehydrocholesterin. Ann. Chem. 520: 98–106.
30. Windaus, A., Schenck, F. & Werder, F. V. (1936) Über das antirachitisch wirksame Bestrahlungsprodukt aus 7-Dehydrocholesterol. Z. Physiol. Chem. 241: 100–103.
31. Velluz, L., Amiard, G. & Goffinet, B. (1955) Le précalciferol. Structure et photochimie. Son role dans le genése di calciferol, et des photoisoméres de l'ergostérol. Bull. Soc. Chim. France 22: 1341–1348.
32. Holick, M. F., MacLaughlin, J. A., Clark, M. B., Holick, S. A., Potts, J. T. Jr., Anderson, R. R., Blank, I. H., Parrish, J. A. & Elias, P. (1980) Photosynthesis of previtamin D_3 in human skin and the physiologic consequences. Science (Washington, DC) 210: 203–205.
33. Windaus, A. & Neukirchen, K. (1919) Die Umwandlung des Cholesterins in Cholansäure. Ber. Dtsch. Chem. Ges. 52: 1915.
34. Bernal, J. D. (1932) Crystal structure of vitamin D and related compounds. Nature (Lond.) 129: 277.
35. Wieland, H. & Dane, E. (1932) Untersuchungen über die Konstitution der Gallensäuren. Z. Physiol. Chem. 210: 268–281.
36. Carlisle, C. H. & Crowfoot, D. (1945) The crystal structure of cholesteryl iodide. Proc. R. Soc. Ser. A 184: 64–82.
37. Butenandt, A. (1961) The Windaus Memorial Lecture. Proc. Chem. Soc. Lond. 1961: 131–138.
38. Deichmann, U. (2002) Chemists and biochemists during the National Socialist era. Angew. Chem. Int. Ed. 41: 1310–1328.

JN THE JOURNAL OF NUTRITION

Erratum

Wolf, G. (2004) The Discovery of Vitamin D: The Contribution of Adolf Windaus. J. Nutr. 134:1299–1302.

The structures of calciferol or vitamin D-2 and cholecalciferol or vitamin D-3 shown in Figure 1, page 1301, are in error. The legend, which was correct, and the revised Figure 1, appear below.

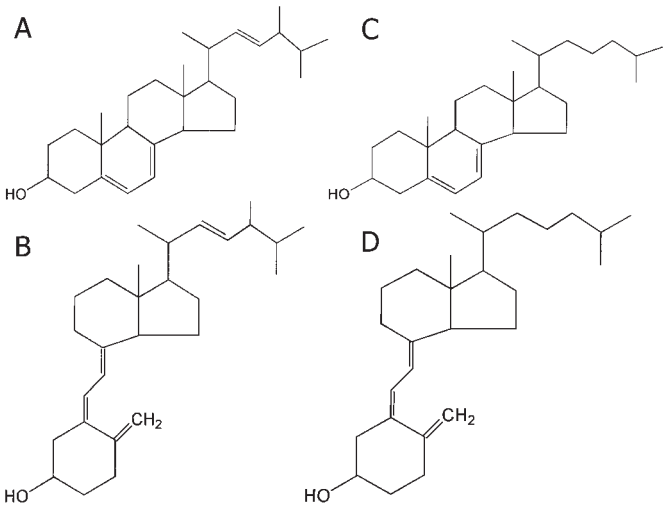

FIGURE 1 Structure of ergosterol (*A*), calciferol or vitamin D-2 (*B*), 7-dehydrocholesterol (*C*), and cholecalciferol or vitamin D-3 (*D*).

0022-3166/04 $8.00 J. Nutr. 134: 2015, 2004.