Vitamin D: Investigations of a New Steroid Hormone

All cells require calcium to function and, since the growth and development of cells is associated with changes in their intracellular calcium concentrations, it has been suggested that calcium may serve as a cellular regulatory agent. Because of the major role that calcium plays in animal physiology, a great deal of interest has been focused on a steroid—derived from vitamin D—that regulates the amount of calcium in an animal's blood and has been called a hormone. Now investigators are beginning to understand how this hormone functions and how its synthesis is controlled. Results from research on this hormone and other metabolites of vitamin D are already being applied to the treatment of a wide variety of human diseases.

Calcium enters the bloodstream when it is actively transported through the walls of the intestine and when it is mobilized from bones. Both of these processes are dependent on the steroid hormone—1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$]—that is metabolized from vitamin D. The hormone is secreted by the kidney and transported to the intestine and bones in response to a skeletal need for calcium. An outstanding question, then, has been: How do an animal's skeletal needs for calcium signal its kidneys to release this hormone?

Many investigators agree that parathyroid hormone is involved in the signal to the kidney to release 1,25-$(OH)_2D_3$ but debate how it is involved. Hector DeLuca of the University of Wisconsin in Madison first showed that rats that lack parathyroid hormone lack 1,25-$(OH)_2D_3$ in their blood. E. Kodicek and Donald Fraser of Cambridge University in England demonstrated that parathyroid hormone apparently affects the enzyme, found in kidney cells, that is responsible for the synthesis of 1,25-$(OH)_2D_3$. They report that the lack of parathyroid hormone in chicken blood is correlated with a decrease in the amount of this enzyme in the kidneys of chickens. These observations have led to conflicting hypotheses of how parathyroid hormone acts on the kidney to stimulate synthesis of 1,25-$(OH)_2D_3$.

Anthony Norman and his colleagues at the University of California at Riverside believe that parathyroid hormone acts directly on kidney cells of chickens and, by extrapolation, other animals, causing them to synthesize the enzyme necessary for the production of 1,25-$(OH)_2D_3$. This effect, they propose, varies directly in proportion to the amount of parathyroid hormone in an animal's blood and inversely in proportion to the amount of vitamin D made available in its diet. They support this conjecture with their own evidence to show correlations between these factors and the presence of the enzyme in kidney cells.

DeLuca proposes an alternative hypothesis—that parathyroid hormone acts indirectly on the kidney by lowering the concentration of phosphate in kidney cells. This lower phosphate concentration, he believes, stimulates the kidney cells to make 1,25-$(OH)_2D_3$. Such a hypothesis, DeLuca notes, can be used to explain the regulation of 1,25-$(OH)_2D_3$ production in a relatively simple way.

The concentration of phosphate, as well as that of calcium, in blood is regulated by 1,25-$(OH)_2D_3$, according to DeLuca. An animal with little 1,25-$(OH)_2D_3$ in its blood would soon have little phosphate in its blood. In such an animal, kidney cells would be stimulated by the low phosphate concentrations to produce 1,25-$(OH)_2D_3$, and this hormone would then act to increase both calcium and phosphate concentrations in the blood. Low calcium concentrations would, concurrently, stimulate parathyroid hormone secretion and this hormone would enhance the mobilization of calcium to the blood.

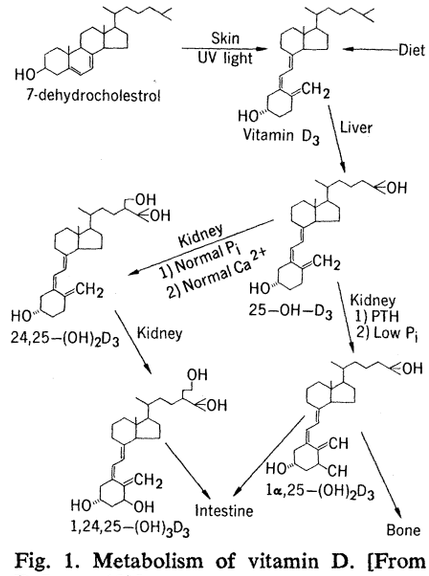

Fig. 1. Metabolism of vitamin D. [From DeLuca (*1*)]

DeLuca's hypothesis about phosphate is based on two observations. First, parathyroid hormone is known to decrease phosphate concentrations in the kidney. Second, dietary phosphate can stimulate 1,25-$(OH)_2D_3$ synthesis in rats even when the animals lack parathyroid hormone. The theory is not universally accepted, however, partly because investigators have not yet been able to show, in animals other than rats, that dietary phosphate regulates 1,25-$(OH)_2D_3$ synthesis.

Since 1,25-$(OH)_2D_3$ is a steroid hormone, investigators have sought to ascertain whether it acts the way other steroid hormones, such as estrogen, testosterone, and hydrocortisone are thought to act. The model for these other steroid hormones is estrogen, whose mode of action has been established by molecular biologists. Estrogen enters a receptive cell, binds to a protein in the cytoplasm, migrates with that protein to the nucleus, binds to the chromatin (that is, the DNA and its associated proteins), and stimulates the cell to synthesize specific proteins. Many investigators attribute the effects of estrogen to its stimulation of protein synthesis.

Direct analogies between the actions of 1,25-$(OH)_2D_3$ and estrogen are proposed by Norman and by Mark Haussler of the University of Arizona College of Medicine, Tucson. Like estrogen, 1,25-$(OH)_2D_3$ binds to a protein when it enters the cytoplasm of a receptive cell, which for this hormone is an intestinal cell. Like estrogen, 1,25-$(OH)_2D_3$ stimulates protein synthesis in intestinal cells (although, according to Fraser, it is likely that only one kind of protein is synthesized by stimulated intestinal cells). And, according to Norman and Haussler, 1,25-$(OH)_2D_3$ binds to the chromatin of intestinal cells to effect this protein synthesis. A chick, rat, or, by analogy, human cell stimulated by 1,25-$(OH)_2D_3$ produces a calcium-binding protein, a function discovered by Robert Wasserman of

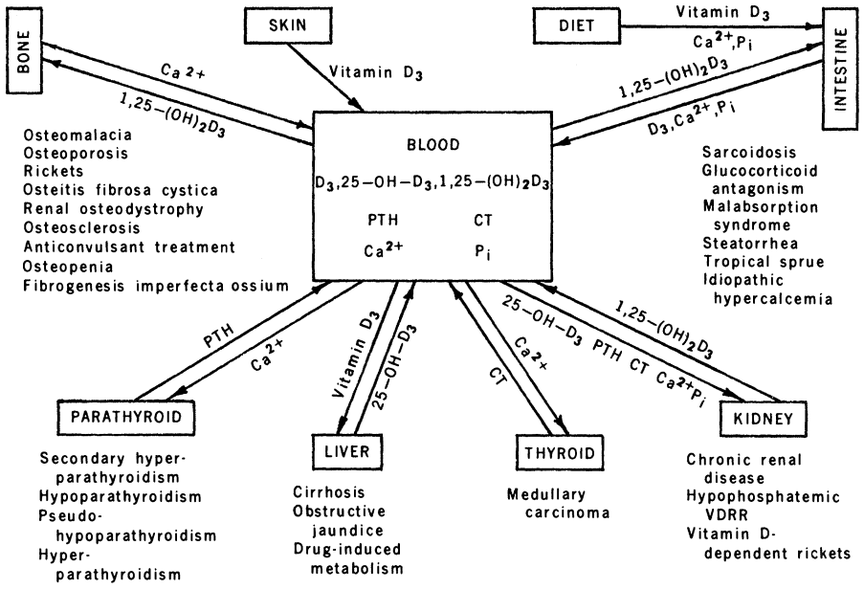

Fig. 2. Human diseases related to vitamin D. [From Norman and Henry (*2*)]

Cornell University, Ithaca, New York. However, the synthesis of this calcium-binding protein is not sufficient to explain the major effect of 1,25-$(OH)_2D_3$ on intestinal cells: the stimulation of calcium transport.

The problem appears to be one of kinetics. The rate that intestinal cells synthesize a calcium-binding protein in response to 1,25-$(OH)_2D_3$ reaches a maximum at about the same time as the rate of calcium transport by these cells reaches a maximum. However, calcium transport falls back to a baseline rate a full 24 hours before the rate of synthesis of calcium-binding protein decreases. Thus calcium transport must require something besides calcium-binding protein. What else is necessary is open to speculation.

Studies of intestinal transport of calcium and control of 1,25-$(OH)_2D_3$ synthesis by the kidney are only the beginning of the research necessary for a new understanding of the role of vitamin D in maintaining the balance of the amount of calcium in the blood.

Vitamin D metabolism is known to involve the liver and the kidneys. Vitamin D is first modified in the liver to 25-hydroxyvitamin D_3 (25-OH-D_3), and then 25-OH-D_3 is transported to the kidneys where it is made into either the steroid hormone 1,25-$(OH)_2D_3$ or one of two other metabolites: 24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2D_3$] or 1,24,25-trihydroxyvitamin D_3 [1,24,25-$(OH)_3D_3$] (Fig. 1). No one knows the molecular mechanisms that control the synthesis of 25-OH-D_3, 24,25-$(OH)_2D_3$, and 1,24,25-$(OH)_3D_3$ or whether these metabolites of vitamin D have any functions or even whether any other metabolites of vitamin D are made. Moreover, no one knows how the steroid hormone 1,25-$(OH)_2D_3$ acts on bones nor how an animal's needs for calcium affect the synthesis of parathyroid hormone. Nonetheless, the very discovery of the sequence of chemical modifications that vitamin D undergoes when 1,25-$(OH)_2D_3$ is produced is proving invaluable to the treatment of human diseases (Fig. 2).

Since vitamin D is first modified in the liver, diseases that affect the liver usually result in defective production of the metabolite 25-OH-D_3 and thus defective production of the hormone 1,25-$(OH)_2D_3$. Several groups of investigators have noticed that anticonvulsant drugs, such as phenobarbital and diphenylhydantoin, apparently affect the synthesis of 25-OH-D_3 by the liver.

Humans who are given these drugs for long periods of time often develop rickets, osteomalacia, or hypocalcemia. Alcoholics, too, suffer diseases of calcium deficiency because of impaired liver function, according to Louis Avioli of Washington University School of Medicine in St. Louis. Avioli and his associates and several other groups of investigators are successfully treating, with large doses of vitamin D, patients with calcium deficiencies that result from malfunctioning livers. According to Avioli, the livers of these patients can still synthesize 25-OH-D_3, although with decreased efficiency. Thus the patients respond to large doses of vitamin D. Large doses of vitamin D are, of course, only useful to patients with specific diseases that affect vitamin D metabolism. For most people, such doses of vitamin D are harmful.

Like those with impaired livers, patients with chronic kidney diseases often develop bone diseases and hypocalcemia. These people cannot synthesize the hormone 1,25-$(OH)_2D_3$. Jack Coburn and his associates at the University of California School of Medicine at Los Angeles, together with Norman, have successfully treated more than 50 patients with renal failure by giving them 1,25-$(OH)_2D_3$.

Hypoparathyroid patients lack parathyroid hormone and fail to make 1,25-$(OH)_2D_3$. Thus they have abnormally low concentrations of calcium in their blood. This results in severe bone disease. DeLuca and his clinical associates have used 1,25-$(OH)_2D_3$ along with calcium to correct deficits in serum calcium concentrations of eight hypoparathyroid patients.

Bone disease is a well-recognized consequence of long-term corticosteroid therapy. Corticosteroids are being given to people with such diseases as rheumatoid arthritis, systemic lupus erythematosis, and asthma as well as to people who receive transplants. Although there is some disagreement as to how these drugs affect vitamin D metabolism, Avioli and his colleagues report that they can treat patients who take corticosteroids by giving them large doses of vitamin D and thereby overcome the adverse effects of these drugs. As with most clinical applications of research on vitamin D, this treatment is relatively independent of a detailed knowledge of the molecular mechanisms that operate in the metabolism of vitamin D. While these molecular mechanisms are now under active investigation, much remains to be learned about the derivatives of vitamin D and their effects on human physiology.—GINA BARI KOLATA

Additional Reading

1. H. E. DeLuca, *Fed. Proc.* **33**, 2211 (1974).
2. A. W. Norman and H. Henry, *Recent Prog. Horm. Res.* **30**, 431 (1974).