REVIEW ARTICLE

J. Sci. Soc. Thailand, 5 (1979) 117-130

CALCIUM HOMEOSTASIS

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(Received 21 June 1979)

The level of calcium in plasma is controlled within a small range close to 10 mg/100 ml. This relatively steady level is maintained not by an inherent stability of various processes of the body but by complex, dynamic mechanisms. The generally accepted concept of calcium homeostasis involves so-called "negative feedback" mechanisms of various interrelated hormonal systems, like parathyroid hormone (PTH), calcitonin (CT), vitamin D metabolites, and organ systems namely: bone, intestine and kidney. Since it is difficult to incorporate all the available data into one theory that adequately explains how a constant level of plasma calcium is maintained in most animals, a more general approach will be taken and each calcium regulating hormone will be briefly discussed.

Calcium is present in the plasma in three forms, ionized, protein bound and complexed with organic anions, e.g., citrate; all adding up to 2.45 mM or 10 mg/100 ml plasma, 65% of which is in the ionized form. It is the ionized calcium (Ca^{2+}) that controls and is controlled by the various hormones and it is this fraction that is involved in all aspects of calcium metabolism. Changes in calcium concentration of cell cytosol regulate functions of membrane and cell organelles, e.g. excitation-contraction coupling of the muscles, exocrine and endocrine secretions, neurotransmitter secretion, hormone action and energy metabolism in a wide variety of cells. In addition, calcium in the form of crystalline structure is vital for the integrity of skeletal system.

Maintenance of calcium level in both extracellular fluids within narrow limits in normal animals despite variations in dietary input and absorption of calcium from the gastrointestinal tract is made possible through the control systems containing negative feedback loops (Figure 1). This controlling system includes the parathyroid glands, the C cells of thyroid gland which secrete calcitonin, the kidney where the active metabolite of vitamin D is formed and other possible calcium regulating hormones. The controlled system includes the extracellular fluid and the three organ systems: the skeleton, kidneys and gastrointestinal tract.

Parathyroid Hormone

Parathyroid hormone (PTH) plays the principal role in calcium homeostasis by maintaining the concentration of Ca^{2+} in the extracellular fluids at a constant level and correcting any fall in plasma calcium. It is secreted from chief cells of the parathyroid glands which consist of small bodies, usually four in number, located near or embedded in the thyroid glands. Active parathyroid extract was first prepared by Collips in 1925[1]. Isolation of bovine and porcine PTH of 98% or greater purity is now possible[2]. The amino acid compositions of human, bovine and porcine PTH are very similar, comprising 84 residues with a total molecular weight of 9500, and with biological activity residing in the initial 30 residues of the amino terminal[2]. The hormone is synthesized in the parathyroid gland as a prohormone of 109 amino acids, with a molecular weight of approximately 12,000. Pro-PTH undergoes the first cleavage which is supposedly a point of metabolic control in the parathyroid cells into 84 residue peptide. Once it is secreted into the circulation, it undergoes a second cleavage probably in the liver into a number of fragments with active fragment containing the N-terminal sequence. PTH is inactivated in the kidney and liver[3,4]. It has been well documented that a reduction in plasma ionized calcium concentration by either EDTA or inorganic phosphate stimulates PTH secretion. However, the storage pool available for immediate release is relatively small so that the enhanced release of PTH observed initially in response to a reduction in plasma Ca^{2+} may fall off despite a further decrease in the Ca^{2+} concentration[5]. The hypocalcemic stimulus to PTH release is mediated by the activation of the adenyl cyclase in the chief cells[6,7]. Thus, the parathyroid gland is unique in that hormone secretion is inhibited by calcium while the secretory activity of most endocrine glands is activated by calcium.

Most of the general physiological actions of PTH are well known and will only be briefly mentioned.

Kidney. PTH increases the renal phosphate excretion by inhibiting the phosphate reabsorption in proximal tubule[8], while decreases urinary calcium excretion by pro moting reabsorption of Ca^{2+} at various sites along the nephron. This PTH action is probably mediated by cyclic AMP (cAMP)[9].

Bone. The experiments of Barnicot[10] and Chang[11] showed that parathyroid glands transplanted to sites immediately adjacent to bone caused resorption of skeletal tissue. These studies as well as those utilizing bone transplants in tissue culture[10-12] indicated that the effect of PTH on bone in tissue culture consists of two phases. The earlier phase is more rapid, manifested by increased release of calcium into the medium within the first 2-3 hours, and is not dependent on protein synthesis. On the other hand, the second phase which involves activation and biosynthesis of new proteins; the latter is blocked by the metabolic inhibitor, actinomycin D.

Intestine. The influence of PTH on gastrointestinal absorption of calcium seems to be indirect via the regulation of vitamin D metabolism in kidney[13].

Several actions of PTH on target tissues could be attributed to a single type of hormone-receptor interaction which results in changes in intracellular concentration of cAMP as suggested by Aurbach[13]. However, recent studies on the mechanism

of action of PTH on embryonic bone *in vitro* indicated that the increased cAMP production is not the exclusive key to each of the physiological effects of the hormone[14]. From the work of Borle[15] which showed an increased entry of calcium into bone after PTH administration, Talmage[16] suggested that immediate physiological action of PTH may be to increase the calcium influx into bone cells and not the increased intracellular concentration of cAMP. The objection to cAMP as the sole mediator of specific PTH action was supported by Hekkelman[17] whose findings suggested that increased cAMP concentration is a general response of cells to certain stimuli. He postulated that certain parameters of PTH action involve the cAMP system which is associated with a low affinity receptor while other parameters involve the calcium, phosphate and citrate release which is probably associated with a high affinity receptor. A model of the action of PTH on bone cell put forward by Hekkelman shows possibility of multiple interrelations between cAMP, other mediators and increased calcium influx, all of which may be affected by PTH and other calcium regulating factors.

Calcitonin

The discovery of calcitionin (CT) in 1961 by Copp[18] has stimulated great interest in the endocrine control of calcium metabolism. By perfusing the thyroid glands of dog with high or low calcium blood, Copp observed that hypercalcemia released a hypocalcemic factor from the gland. But it was not until 1963 that CT was extracted for the first time[19]. The hormone is a polypeptide comprising 32 amino acid residues with a molecular weight of approximately 3500. Inactivation probably occurs in the liver and kidney. In contrast to PTH, the basal secretory rate of CT (10 pmole/kg body weight/hr) is low compared to the hormone content of the thyroid glands (2 nmole/kg body weight) but after stimulation, CT secretion rate can increase 10-100 folds. It has been established that an adenyl cyclase system is involved in the release of CT from thyroid C cells[20,21]. CT secretion has been shown to be affected by several hormones, e.g. glucagon[22,20], epinephrine[23], a C-terminal tetrapeptide common to many gastrointestinal hormones like pancreozymin-cholecystokinin, gastrin and pentagastrin[24]. Endogenous gastrin, enteroglucagon and PZ-CCK stimulated in response to normal physiological stimuli have also been shown to stimulate CT secretion[25].

The hypocalcemic effect of CT can be explained simply as a result of inhibition both of spontaneous bone resorption and PTH or vitamin D stimulated resorption[26,27]. However, Talmage proposed that early effects of CT are more likely to be due to the reduction of calcium efflux from bone cells and bone fluid since the bone resorption requires too long a time to account for the rapid hypocalcemic effect[28]. Furthermore, they postulated that the hypocalcemia produced by CT is secondary to the formation of a calcium phosphate complex in and around osteocytes and lining cells. It is suggested that this complex reduces the availability of calcium for rapid transport to the extracellular fluid, thus resulting in a rapid and transient hypocalcemia[29,30]. Despite controversies on mechanism of action of CT, it has primarily been considered as a hypocalcemic agent which functions by reducing the rate of bone resorption. Its action is further complicated by being age dependent. Kalu[31] observed

that an immediate increase in plasma calcium after thyroparathyroidectomy preceded the subsequent fall in mature rats (weighing over 360 g), whereas in immature rats (50 g) the fall was prompt and progressive. These results thus indicate that in old animals the decrease in the level of circulating CT has a more profound immediate effect than the concomitant decrease in the ambient parathyroid hormone level. On the other hand, immature rats exhibited a prompt drop in plasma calcium after TPTX, suggesting that the importance of the effect of PTH on bone resorption is relatively greater than CT in the acute regulation of plasma calcium in young animals. Later, Gray and Munson[32,33] proposed that the physiological function of CT may be to protect against hypercalcemia after oral ingestion of calcium. By examining this protective role of CT in lactating rats, Toverud[34] found that in contrast to the intact lactating rats, the acutely thyroparathyroidectomized lactating rats showed a marked increased in serum calcium of over 5 mg/100 ml. Experiments in pigs showed similar results. Thus, the results in pigs and rats clearly demonstrated a protective function of CT against postprandial hypercalcemia. This concept is further supported by the fact that CT release can be stimulated by various gastrointestinal hormones[35]. Moreover, Milhaud *et al.*[36] have reported a diurnal variation in serum calcium in rats associated with feeding behaviour. The serum calcium was higher during the unfed hours of light and lower in fed hours of darkness. Acute thyroidectomy during fed hours resulted in a higher serum calcium and loss of the diurnal variation. The interesting point is that the change in the serum calcium pattern preceded the feeding time by an hour or more[37], suggesting a circadian rhythm. This increased CT secretion with expectation of feeding would prevent hypercalcemia at times when there was a perturbation in calcium homeostasis during intestinal calcium absorption.

Gastrointestinal Hormone

Recently, Cooper *et al.*[38] and Care *et al.*[20,23,24] independently proposed a hypothesis suggesting that the presence of calcium in the gastrointestinal (GI) tract or its absorption might have caused secretion of GI hormone which in turn stimulates the release of CT. This hypothesis was supported by the finding that administration of a small dose of pentagastrin, a synthetic pentapeptide containing the biologically active portion of the native hormone gastrin, resulted in a marked, rapid, transitory increase in CT secretion in pig[39,40]. Furthermore, infusion or injection of pentagastrin produced hypocalcemia before, but not after functional thyroidectomy by which venous blood leaving the thyroid gland was not allowed to enter the systemic circulation. Blood phosphate also fell. These results suggested that the hypocalcemia and hypophosphatemia observed when the thyroid circulation was intact, occurred in response to an elevated secretion of pentagastrin induced CT secretion. Beside pentagastrin, another GI hormone, cholecystokinin (CCK) was also found to be a potent secretagogue of CT[41].

The studies mentioned above pointed out that native GI hormones like gastrin and CCK, by stimulating CT secretion, may participate in restricting hypercalcemia after feeding and during intestinal absorption of calcium. However, controversies

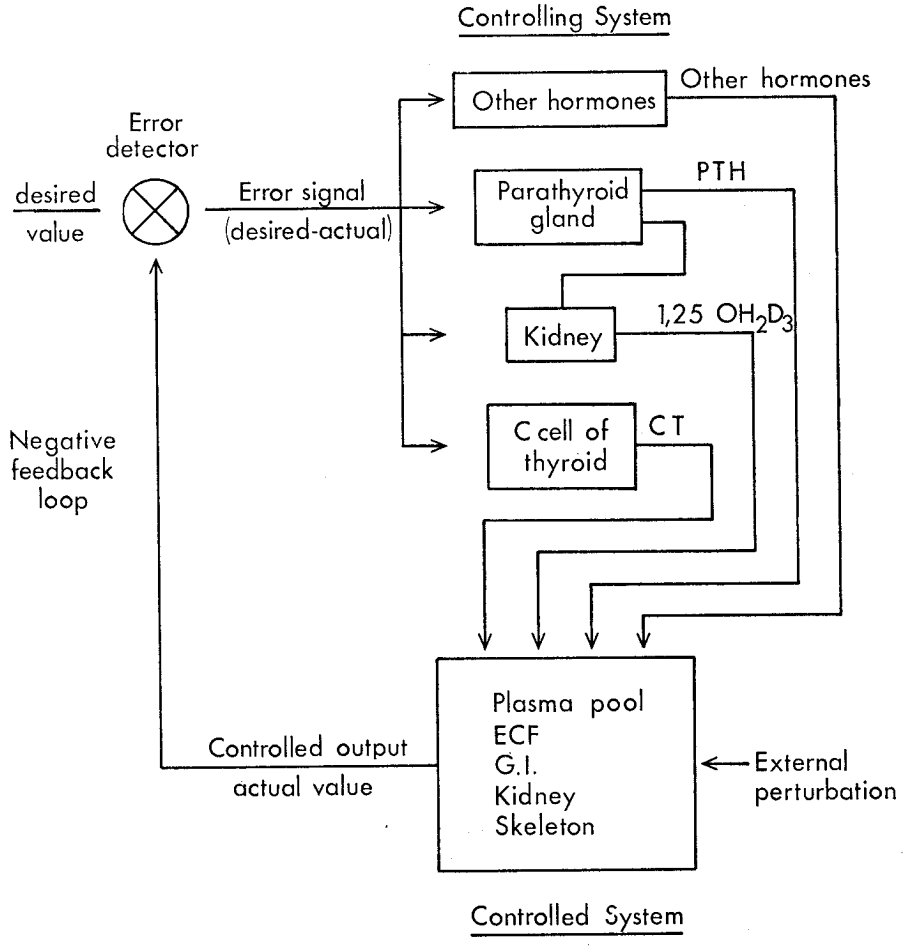

Fig. 1. Block diagram of closed loop control system for calcium. PTH = parathyroid hormone; 1,25 OH_2D_3 = 1,25 dihydroxycholcalciferol; CT = calcitonin; ECF = extracellular fluid; GI = gastrointestinal system.

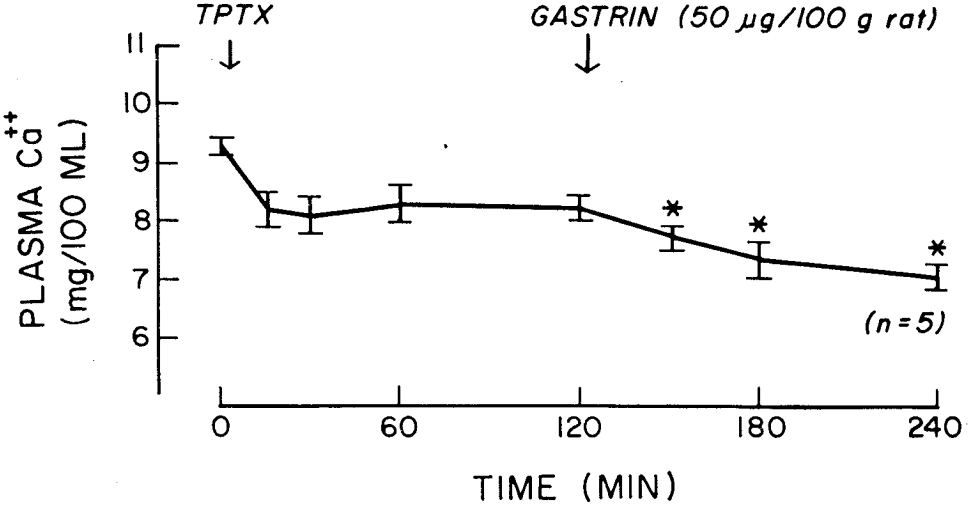

Fig. 2. The effect of thyroparathyroidectomy (TPTX) and the subsequent gastrin administration (50 µg/100 g rat) at the time indicated by an arrow (2 h later), on the plasma Ca++ concentration. *indicates the values of plasma Ca^{2+} after gastrin which are significantly different from the values of plasma Ca^{2+} 2 h after TPTX, ($P<0.05$).

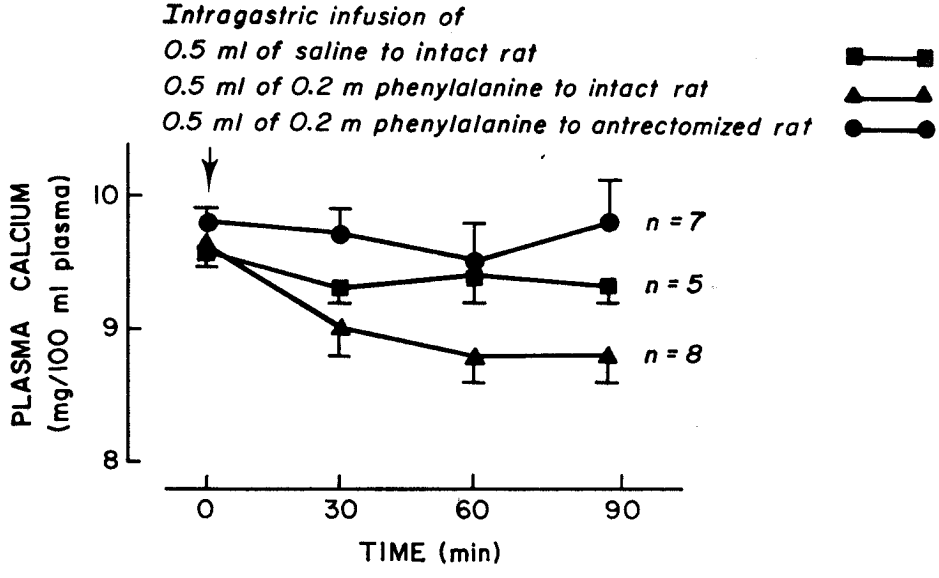

Fig. 3. The effect of endogenous gastrin released by intragastric infusion of phenylalanine upon plasma Ca^{2+}. Phenylalanine in antrectomized rats did not show any hypocalcemic effect when compared to the saline control whereas phenylalanine in the intact rat shows significant hypocalcemic effects at all time intervals tested when compared to either saline control or the phenylalanine in antrectomized group ($P < 0.05$).

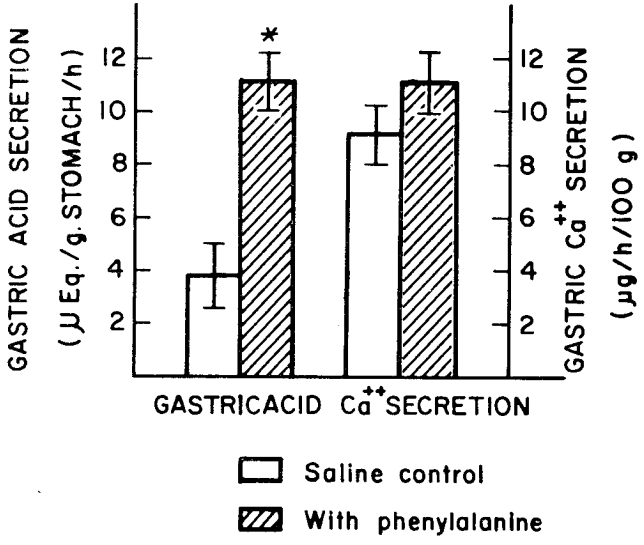

Fig. 4. The rate of gastric acid (μeq/g stomach/h) and gastric calcium secretions (μg Ca^{2+}/100 g rat/h) in response to intragastric infusion of phenylalanine into the intact rats (n = 6). The control animals were intragastricly infused with normal saline (*indicates $P < 0.001$).

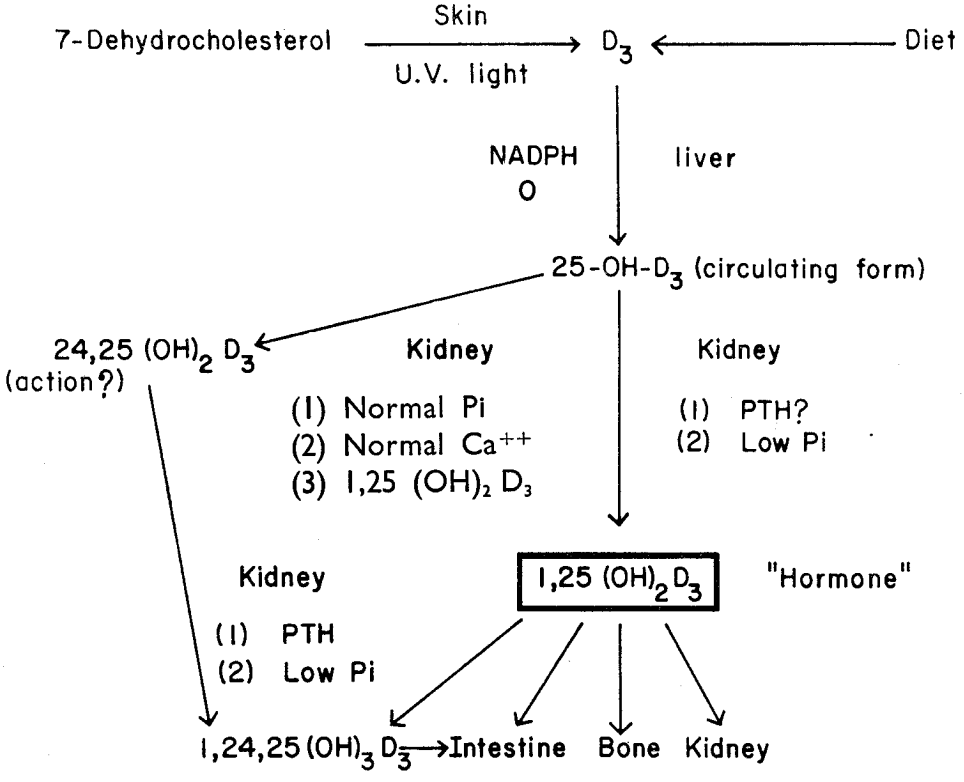

Fig. 5. The functional metabolism of vitamin D.

arose when Schulak *et al.*[42] and Krishnamra *et al.*[43] were able to show that gastrin-induced hypocalcemia could be demonstrated both in intact and thyroparathyroidectomized rats (Figure 2). On the basis of their findings, it was suggested that at least in the rat, mechanisms other than the release of CT was possible.

Since the results describes above were from pharmacological doses of gastrin, the physiological role of gastrin as a hypocalcemic agent has now been established by Krishnamra (unpublished data) i.e., the hypocalcemic effect of gastrin was again elicited by the endogenous release of the hormone induced by amino acid phenylalanine infused into the gastric antrum. The antrum of the pyloric gland region of the stomach is the major and most abundant source of gastrin which is normally released by neuroendocrine reflexes and a few chemicals like short chain alcohol[44], amino acids, especially glycine, alanine, cysteine and phenylalanine[45,46]. Figure 3 clearly shows that intragastric infusion of phenylalanine into the intact rats causes hypocalcemia whereas saline infusion into intact rat and phenylalanine into antrectomized rat did not. The releases of endogenous gastrin was inferred by the fact that phenylalanine infused into the intact rats also caused concomitant increase in acid secretion (Figure 4).

Thus the above studies suggest that gastrin may constitute another calcium control system, especially in the case of excess Ca absorption. Nevertheless, this protective function of GI hormones has not been demonstrated in other mammalian species and its mechanism has yet to be elucidated. Thus, the concept that protection against hypercalcemia is one of the important physiological functions of the GI hormones has not yet been accepted.

Vitamin D

A great change in thought on calcium metabolism is being brought about by new data on the metabolites of vitamin D, and the realization that, via its metabolites, vitamin D is a very important hormone. It was in 1970 that Fraser and Kodicek[47] found the biosynthesis by kidney of the most biologically active vitamin D metabolite, 1,25-dihydroxy cholecalciferol [1,25 (OH) $_2D_3$]. So it becomes clear that the vitamin is but the prohormone of an extremely potent steroid secreted by the kidney. This is the true hormone which plays a major role in calcium regulation. A summary of what is currently known regarding the pathway of vitamin D metabolism is illustrated in Figure 5.

Briefly, vitamin D from the diet or from the irradiation of 7-dehydrocholesterol in the skin accumulates in the liver where it undergoes the first metabolic alteration. Here vitamin D is converted to 25-OH-D_3 by a reaction requiring NADPH and molecular oxygen[48]. 25-OH-D_3, the major metabolite circulating in the blood, is bound to an α_2-globulin in plasma and transported to the kidney where it undergoes a second obligatory hydroxylation to the active metabolite 1,25 $(OH)_2D_3$. This step takes place in the mitochondria of the renal cells at least in the chick[47,49]. It is this form which stimulates intestinal transport of calcium and phosphate, bone calcium

mobilization and other reactions attributed to the function of vitamin D[50]. Under normal phosphate and calcium state, 25-OH-D_3 may be hydroxylated to 24,25-$(OH)_2D_3$ which is a less active metabolite whose action is not fully understood. However, PTH or low level of inorganic phosphate will stimulate the conversion of 24,25 $(OH)_2D_3$ to 1,24,25 $(OH)_3D_3$ which has an effect on intestinal calcium transport in the rat.

The reaction that is controlled physiologically is the hydroxylation of 25-OH-D_3 to 1,25 $(OH)_2D_3$. In the past few years, there has been an extensive study involving factors governing the biosynthesis of 1,25 $(OH)_2D_3$. In 1971, Boyle, Gray and DeLuca[52] demonstrated that the production and appearance of 1,25 $(OH)_2D_3$ in the rat is markedly stimulated by the feeding of low calcium diet and is suppressed in animals given a high calcium diet. Low phosphate diet was also shown to result in a dramatic increase in the output of 1,25 $(OH)_2D_3$[52]. It was postulated that this dietary effect on the production of vitamin D metabolites was mediated via PTH and indeed it was suggested that PTH is the essential trophic factor regulating the production of 1,25 $(OH)_2D_3$ This has been substantiated by some groups[53,54], while contradicted by others[55,56]. Therefore, the physiological role of PTH on vitamin D metabolism remains to be clarified.

Actions of 1,25$(OH)_2D_3$, Active Vitamin D Metabolite

Intestine. The best documented effect of vitamin D is the role in facilitating calcium absorption from the intestine. It is apparent that vitamin D stimulates a phosphate transport system in the intestine that is distinct from the calcium transport process[57]. Like other steroid hormones, 1,25$(OH)_2D_3$ exerts its effect via RNA and protein synthesis through a receptor system identified by Brumbaugh and Haussler[58, 59] for 1,25$(OH)_2D_3$ in chick intestine that apparently serves the purpose of translocating the hormone to the nucleus. As a result of 1,25$(OH)_2D_3$ entering into the cell, two specific proteins have been detected. The first is a phosphatase enzyme, alternately described as an alkaline phosphatase[60] and as a calcium ATPase[61]. The kinetics of appearance of this protein after administration of 1,25$(OH)_2D_3$ are slower than the increase in calcium absorption implying that this protein may not be involved in calcium transport but may participate in other vitamin D effects. A second possible specific protein, 2% of total protein in chick intestine, is the calcium binding protein (CaBP)[62]. This protein is absent in vitamin D deficient animals and fluctuates in concert with level of calcium transport. However, the observation that the decay of calcium transport occurs more quickly than the disappearance of CaBP[62] and that the localization of CaBP is outside the microvillar membrane suggests that CaBP is not the only component of the induced calcium transport system in intestine. According to a hypothetical model proposed by Zerwekh[63], 1,25$(OH)_2D_3$ stimulates the synthesis of CaBP and possibly other functional protein (FP). The newly synthesized CaBP and FP are then secreted into the intestinal lumen from goblet cell and become associated with the secreted mucus. CaBP binds free calcium and the complex becomes associated with the microvillar brush border membrane of the absorptive cell. With

possible involvement of FP, the membrane permeability is modified and calcium enters the cell.

Bone. Bone is a second tissue at which vitamin D acts to moboilize calcium for the circulation. This response to the hormone requires RNA synthesis[64]. Regarding the involvement of PTH in $1,25(OH)_2D_3$ mediated calcium resorption, it is well accepted that this process does not require PTH in organ culture[65,66] but conflicting results have been obtained from bone resorption *in vivo* experiments[67,68].

Kidney. Regarding the kidney, vitamin D dependent CaBP has been detected in chick[62] and human kidney[69]. There seems to be conflicting data concerning the physiological important of vitamin D in kidney. On one hand, 99% of calcium filtered by the kidney is reabsorbed even in vitamin D deficiency, on the other hand, $1,25(OH)_2D_3$ has been reported to increase renal reabsorption of calcium[70,71]. So this effect of vitamin D remains to be investigated.

Of the many postulations and proposed models for the mechanism of action of the various hormones mentioned, how much is inaccurate and how much will finally be proved to be fact is purely a matter of conjecture at present. Nevertheless, they seem to be consistent with the currently available data and so provide a framework for the design of further experiments.

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