Endocrine Control of Calcium Metabolism and Parturient Hypocalcemia in Dairy Cattle

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Calcium plays a key role in many fundamental biologic processes including neuromuscular excitability, membrane permeability and blood coagulation in addition to being an essential structural component of the skeleton. The precise control of calcium ions in extracellular fluids is vital, therefore, to the health of animals and man. To maintain a constant concentration of calcium, despite marked variations in intake and excretion, endocrine control mechanisms have evolved that primarily consist of the interactions of three major hormones. Although the direct roles of parathyroid hormone, calcitonin and vitamin D frequently are emphasized in the control of blood calcium, other hormones such as adrenal corticosteroids, estrogens, thyroxine, somatotrophin and glucagon may contribute to the maintenance of calcium homeostasis under certain conditions. The objectives of this report are: (a) to summarize and illustrate some recent findings on the structure and function of calcium control mechanisms under normal and pathologic conditions; and (b) to relate these findings where indicated to the pathogenic mechanisms of parturient hypocalcemia and paresis in dairy cattle.

Parathyroid Hormone

The parathyroids in cows and most other animal species consist of two pairs of small glands located in the anterior cervical region. Present evidence indicates the parathyroid glands are composed of a single basic cell type that exists in different stages of secretory activity and are concerned with the elaboration of one hormone. Parathyroid hormone is a straight chain polypeptide consisting of 84 amino acid residues (7). The hormone is synthesized within chief cells by ribosomes attached to the endoplasmic reticulum, concentrated and packaged in the Golgi apparatus, and stored within membrane-limited secretion granules (Figure 1). A larger precursor molecule (prohormone) may be synthesized by chief cells from which biologically active parathyroid hormone is cleaved prior to or after secretion (3,24). In response to the stimulus of hypocalcemia storage granules migrate to the peripheral

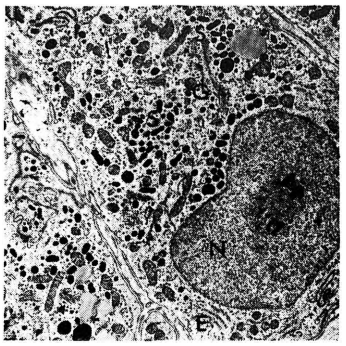

Figure 1: Electron micrograph illustrating a secretory (chief) cell in the bovine parathyroid gland. In normal cows the dense secretions (S) granules (intracellular storage form of parathyroid hormone) are distributed throughout the cytoplasm. The endoplasmic reticulum (E) with attached ribosomes represents the site of hormonal synthesis and the Golgi apparatus (G) is concerned with the packaging of hormone into storage granules. N = chief cell nucleus. Magnification X 4,500. From Capen (9), by permission of R. H. Donnelley Corporation.

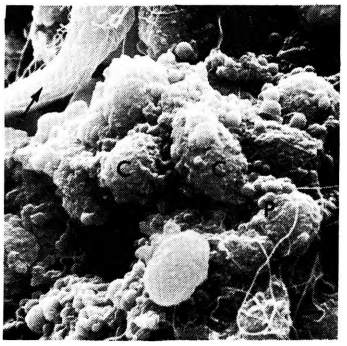

Figure 2: Scanning electron micrograph illustrating the surfaces of a group of parathyroid chief (C) cells. Fine (arrow head) and dense (arrow) collagen fibers join groups of cells. Note the numerous spherical projections (P) from the surfaces of the chief cells, many of which contain secretion granules of parathyroid hormone. Magnification X 2,600.

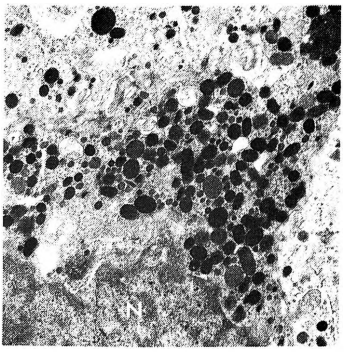

Figure 3: Suppressed secretory cell in the parathyroid of a cow receiving vitamin D prepartum. As a response to the hypercalcemia induced by vitamin D, chief cells accumulate storage granules since the stimulus for parathyroid hormone secretion is diminished. N = chief cell nucleus. Magnification X 7,500. From Capen (9), by permission of R. H. Donnelley Corporation.

portions of chief cells, fuse with the plasma membrane, and are extruded from chief cells within cytoplasmic projections (Figure 2). Conversely, when the stimulus for secretion of hormone is diminished by hypercalcemia, such as induced by large doses of vitamin D, chief cells accumulate storage granules and secretory organelles become atrophic (Figure 3) (13). Parathyroid hormone increases bone resorption by stimulating osteocytic and osteoclastic osteolysis, mobilizes calcium from skeletal reserves, and returns the blood concentration of calcium toward normal. It also acts to increase phosphate clearance by diminishing renal tubular reabsorption (2).

Initial studies on parturient hypocalcemia in our laboratory were designed to test the hypothesis, proposed by other investigators (21),

that the fundamental deficit responsible for the decrease in blood calcium and phosphorus was an inability of the parathyroid glands to respond appropriately in order to meet the increased demands for these ions near parturition. The secretory activity of parathyroids from cows with parturient hypocalcemia was evaluated ultrastructurally and compared to control parturient cows (10). Glands of diseased cows appeared to be fully capable of responding to the increased demands by secretion of stored hormone and hypertrophy of secretory organelles for synthesis of new hormone. Parathyroid cells either were depleted of storage granules or the granules had migrated peripherally and were fused with the plasma membrane (Figure 4). These structural findings suggested an active secretory response by parathyroid glands in cows with parturient hypocalcemia and were in agreement with the excellent biochemical studies of Mayer et al. (30). They utilized a sensitive immunoassay to measure plasma levels of parathyroid hormone and detected equal or elevated levels of hormone in cows with parturient paresis compared to control parturient cows. Therefore, the ability of parathyroid glands to respond to the challenge for extra calcium mobilization with increased hormonal secretion and synthesis does not appear to be defective in cows that develop parturient hypocalcemia and paresis.

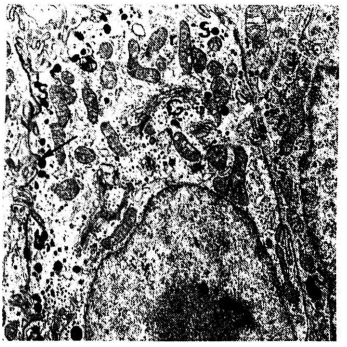

Figure 4: Hyperactive chief cell in the parathyroid gland of a cow with parturient hypocalcemia and paresis. In response to the increased demand for parathyroid hormone, secretion granules (S) have migrated peripherally and many are fused with the plasma membrane (arrow) of the cell. Secretory organelles such as the Golgi apparatus (G), ribosomes (r), and mitochondria (M) are hypertrophied in these actively secreting cells. Magnification X 6,100. From Capen and Young (10), by permission of Williams and Wilkins Company.

Calcitonin

Calcitonin (thyrocalcitonin) is a recently discovered hormone (18) which is synthesized and secreted by parafollicular (C-) cells of the mammalian thyroid gland (22). Parafollicular cells are derived from primordia of neural crest origin that migrate into the ultimobranchial body during early embryonic development prior to its incorporation into the thyroid gland (32). Calcitonin is a smaller molecule than parathyroid hormone consisting of 32 amino acid residues with a 1-7 intrachain disulfide linkage (17). It is secreted continuously under normal conditions and its potent hypocalcemic and hypophosphatemic effects primarily are the result of a temporary inhibition of parathyroid hormone-stimulated bone resorption (1,23,34). The physiologic function of calcitonin has been suggested to be the protection against postprandial hypercalcemia (26) and the prevention of excessive

demineralization of the skeleton by parathyroid hormone and other factors (33). Therefore, calcitonin and parathyroid hormone acting in concert provide a dual feedback mechanism to maintain the concentration of calcium within precise limits.

Parafollicular cells in normal cows were located at the periphery of thyroid follicles and extended cytoplasmic processes between thyroxine-producing follicular cells (Figure 5). The secretory polarity was directed toward interfollicular capillaries and the large cytoplasmic area contained numerous secretion granules (10). These storage granules were composed of fine dense particles, surrounded by a limiting membrane, and have been shown by other investigators (4) to contain the calcitonin activity of parafollicular cells (Figure 6). Parafollicular cells store more hormone in the form of secretion granules for rapid secretion than chief cells of the parathyroid gland (9). Hypercalcemia is known to be the primary stimulus for calcitonin secretion (14). Parafollicular cells respond initially to hypercalcemia by a discharge of storage granules and subsequently by hypertrophy of organelles concerned with new hormone synthesis (Figure 7) (11).

Subsequent investigations were designed to investigate the role of calcitonin in the pathogenesis of parturient hypocalcemia. Thyroid extracts from diseased cows had a consistent and significant (p 0.001) reduction in calcitonin content (83 $\pm$ 23 MRC mU/g) to 14% of control cows (614 $\pm$ 172 MRC mU/g) (39). Many parafollicular cells were degranulated and appeared to have discharged much of their stored

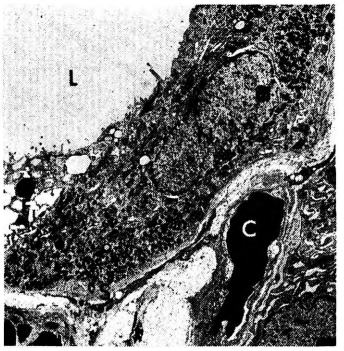

Figure 5: Calcitonin-secreting parafollicular (C-) cell in the wall of a thyroid follicle from a parturient control cow. The cytoplasm contains numerous hormone-containing secretion granules (S). The secretory polarity of C-cells is directed toward the interfollicular capillaries (C) whereas thyroxine-producing follicular cells (F) are orientated toward the luminal colloid (L). N = nucleus of parafollicular cell. Magnification X 2,700.

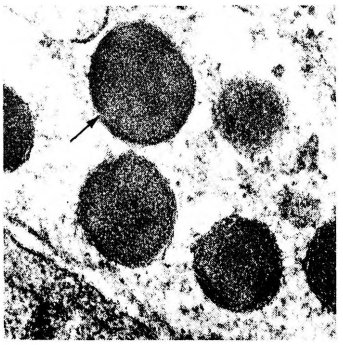

Figure 6: Electron micrograph of a calcitonin-containing secretion granule in a C-cell from a parturient control cow. These storage granules are composed of numerous dense particles and surrounded by a single limiting membrane (arrow). Magnification X 64,400.

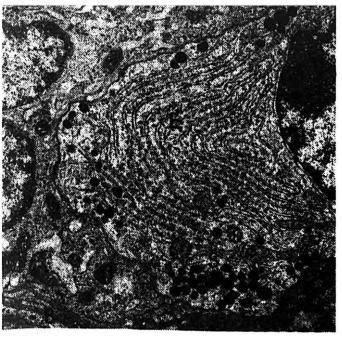

Figure 7: Hypertrophic parafollicular cell from a cow receiving vitamin D (30 million units daily for five days). In response to hypercalcemia the protein synthetic apparatus (E) is extensive and storage granules (S) are situated peripherally in the cell. B = basement membrane of thyroid follicle. Magnification X 7,250.

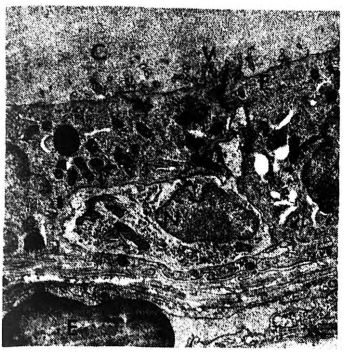

Figure 8: Degranulated parafollicular cell in the wall of a thyroid follicle from a cow with parturient hypocalcemia and paresis. Parafollicular cells of diseased cows have only a few secretion granules (S) and thyroidal extracts contain only 14% as much calcitonin activity as control cows. N = nucleus of parafollicular cell. Follicular cells (F) with microvilli (V) border the luminal colloid (C). An interfollicular capillary with an erythrocyte (E) is present at the bottom. Magnification X 9,450. From Capen and Young (10), by permission of Williams and Wilkins Company.

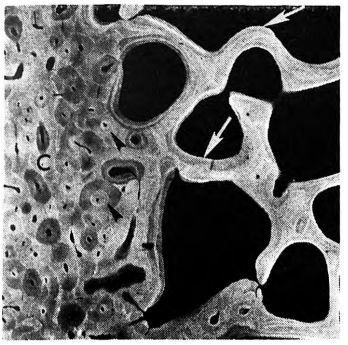

Figure 9: Microradiograph of a section from the rib of a cow with parturient hypercalcemia and paresis illustrating the predominately inactive surfaces in trabecular and compact bone. Inactive bone surfaces (arrows) are smooth and bordered by a line of increased radiodensity. The compact bone (C) at the left is composed of closed osteons of varying density with "halo" formation (arrow heads) in several osteons. Magnification X 16.5. From Rowland et al. (35), by permission of Springer-Verlag.

secretory product (Figure 8). Studies by others have suggested that parturition in cows was associated with relatively high plasma calcitonin levels and that calcitonin secretion at this time seemed to escape from direct control by blood calcium (15). Elevated plasma levels of immunoreactive calcitonin have been reported in cows prior to the development of profound hypocalcemia by some investigators (28) but not by others (29). These findings suggest that secretion of calcitonin prepartum in certain cows may be one factor that contributes to the inability of increased parathyroid hormone levels to mobilize calcium rapidly from skeletal reserves and maintain blood levels during the critical period near parturition. The stimulus for calcitonin secretion in cows is uncertain but may be related to a long term excess of calcium in prepartal diets.

Since the major target cells of both parathroid hormone and calcitonin are in bone, matrix catabolism and bone structure were evaluated in cows with parturient paresis compared to control parturient cows (35). Bone turnover, particularly resorption, was low compared to either pre- or postpartal control cows. The percentage of trabecular and Haversian bone surfaces on microradiographs undergoing resorption in diseased cows (3.39 $\pm$ 0.73%) (Figure 9) was similar to that of nonpregnant-nonlactating control cows (3.30 $\pm$ 0.05%). There was not an excessive accumulation of osteoid on bone surfaces that would interfere with the osteoclastic response to parathyroid hormone in cows with parturient hypocalcemia (Figure 10). The urinary excretion of hydroxyproline did not increase significantly during the last month of gestation in cows that developed the disease as occurs in control cows which maintained their serum calcium near normal through parturition and during early lactation (5). The skeletal calcium reserves in cows that developed severe hypocalcemia appeared to be relatively refractory to stimuli which normally increase matrix catabolism. Lactation in control cows was associated with a three-fold increase in bone resorption from seven to ten days postpartum, particularly along trabecular surfaces (Figure 11).

Vitamin D

The third major hormone concerned with calcium homeostasis is vitamin D. The calcemic effects of vitamin D are the result of its

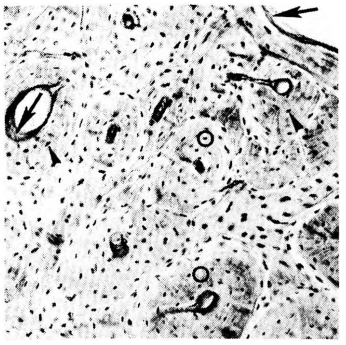

Figure 10: Undecalcified rib section from a cow with parturient hypocalcemia and paresis illustrating closely packed osteons (O) with small osteocytic lacunae (arrow heads) and bone surfaces (arrows) lined by a thin layer of connective tissue. The osteoclastic response to parathyroid hormone in diseased cows was not interfered with by an excessive accumulation of osteoid on bone surface. Magnification X 65. From Rowland et al. (35), by permission of Springer-Verlag.

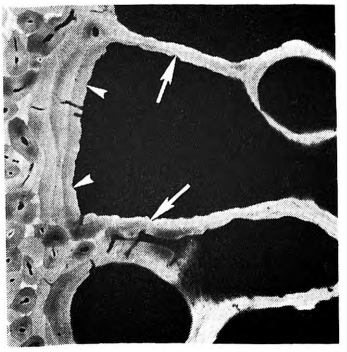

Figure 11: Microradiograph of rib from a postparturient control cow illustrating extensive resorption along trabecular surfaces. Bone surfaces undergoing resorption are irregularly roughened due to the osteoclastic lacunae (arrows) and radiodense. The inner circumferential lamellae are prominent and endosteal surfaces (arrow heads) undergoing resorption are irregular. Magnification X 17. From Rowland et al. (35), by permission of Springer-Verlag.

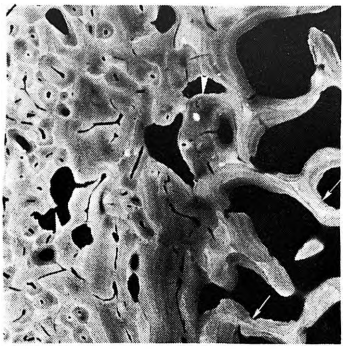

Figure 12: Microradiograph of rib illustrating vitamin D-induced bone resorption in an adult nonlactating cow. Cortical bone has increased porosity due to large resorption spaces (arrow heads) and trabecular surfaces have irregular areas of resorption (long arrow). Magnification X 17. From Rowland et al. (35), by permission of Springer-Verlag.

synergistic actions of increasing bone mineral mobilization (Figure 12) and intestinal calcium absorption (20). Recent findings have established that vitamin D must be metabolically activated before it can produce its known physiologic functions. For example, vitamin D_3 is enzymatically hydroxylated by the liver to 25-hydroxycholecalciferol which is the major circulating metabolite. This first metabolite is further converted by mitochondrial enzymes in the kidney to 1,25 dihydroxycholecalciferol. The synthesis of 1,25 dihydroxycholecalciferol in animals is facilitated by hypocalcemia and the feeding of low calcium diets (6). This biologically active form of vitamin D acts on target cells in the intestine and bone to enhance the rates of existing reactions and increase calcium mobilization. Its onset of action is more rapid and the degree of potency is much greater than vitamin D_3.

Vitamin D should no longer be visualized as a vitamin but rather as a hormone (19). It is not required in the diet since animals under normal conditions can synthesize vitamin D from precursor molecules by photoactivation if they have access to sunlight. The liver and kidney function as endocrine organs to synthesize the active forms of vitamin D. The mechanism of action by binding to nuclear receptors and stimulating protein synthesis plus the chemical structure of the biologically active form of vitamin D are similar to other steroid hormones. Also, the known toxic effects of large doses of vitamin D, such as used to prevent parturient paresis (12), are more characteristic of hormones than of most vitamins.

Vitamin D-mediated calcium absorption occurs through the integrated function of two populations of cells present in the intestine. The projection of villi from the floor into the lumen of the intestine greatly increases the effective surface area for absorption of nutrients (Figure 13). Intestinal absorptive cells are responsive to vitamin D and are concerned with the transport of calcium from the lumen to the blood stream. The luminal surface (brush border) of absorptive cells is highly specialized and has numerous microvilli which further increase the intestinal surface area (Figures 14-16). The trilaminar membrane of the microvilli (Figure 16) contains vitamin D-dependent enzymes (e.g. Ca-ATPase) thought to be concerned with the translocation of calcium

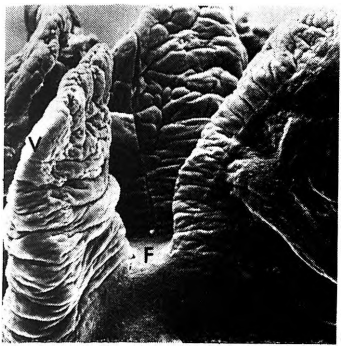

Figure 13: Scanning electron micrograph illustrating the structure of the small intestine where absorption of calcium occurs from dietary sources. Villi (V) project from the floor (F) of the intestine into the lumen and greatly increase the surface area. The surfaces of intestinal villi appear relatively smooth at low magnification. Magnification X 100.

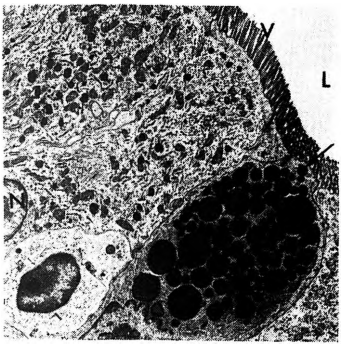

Figure 14: Section through an intestinal villus illustrating the two types of cells that are responsive to vitamin D and are concerned with calcium absorption. The surface of the tall absorptive cells (N = nucleus) has numerous microvilli (V) projecting into the intestinal lumen (L) and large mitochondria in the cytoplasm. Absorptive cells are concerned with the transport of calcium from the lumen to the blood stream. A goblet cell with large secretion granules (S) opens into the lumen (arrow) between two absorptive cells. Goblet cells secrete a calcium-binding protein onto the luminal surface that sequesters calcium near the microvilli. Magnification X 2,700.

Figure 15: Surface architecture of the intestinal villi illustrated in Figure 13 at higher magnification. Junctions (arrow heads) between individual absorptive cells and orifices of goblet cells (G) can be visualized. The luminal surface of absorptive cells is modified by the presence of numerous microvilli (arrows) which further increase the intestinal surface area. Magnification X 2,500.

Figure 16: Microvilli of absorptive cells extending into the intestinal lumen (L). The trilaminar membrane (arrow heads) of the microvilli contains vitamin D-dependent enzymes thought to be concerned with the translocation of calcium into absorptive cells. Calcium-binding protein secreted by goblet cells sequesters calcium near these microvillar membranes. Fine filaments (arrow) in the cores of microvilli extend to the terminal web (T) of absorptive cells. Magnification X 15,800.

from the lumen into absorptive cells (20). The transcellular transport of calcium from the luminal to the basilar aspect of the cell probably is mediated by the numerous mitochondria in the cytoplasm.

Goblet cells of the small intestine are the second group of cells which contribute to calcium absorption. They are located in the intestinal mucosa wedged between absorptive cells and in response to vitamin D synthesize and secrete a specific calcium-binding protein (CaBP) (36,37) which becomes incorporated into the surface coat (glycocalyx) of the intestinal brush border (Figure 15). The apical portion of the cytoplasm of goblet cells is filled with large membrane-limited granules that are extruded through small orifices onto the luminal surface (Figures 14 and 15). The absorptive capacity of the intestine for calcium is a direct function of the amount of CaBP present (27,38). The administration of vitamin D or feeding low calcium diets, two methods used to prevent parturient paresis in cows, stimulates the synthesis of CaBP by goblet cells in other species thereby increasing the intestinal absorption of calcium. The physiologic function of CaBP may be related to an ability to sequester dietary calcium from the intestinal lumen in close proximity to microvillar membranes thereby facilitating the enzymatic translocation of calcium into absorptive cells.

Vitamin D in pharmacologic doses (20 to 30 million units/day) immediately prior to parturition has been used to reduce the incidence of parturient paresis (25). Cows receiving vitamin D have morphometric evidence of increased bone resorption in both cortical and trabecular bone (Figure 12) (35), depleted thyroid stores of calcitonin (39), a larger exchangeable calcium pool (31), and increased calcium absorption from the gut (16), probably through an activation of one or more components of the intestinal calcium transport system. However, the administration of massive doses of vitamin D is not without toxic effects. Cows receiving vitamin D for 10 days or longer develop widespread cardiovascular mineralization and nephrocalcinosis (12). Preliminary evidence suggests that the administration of more potent and rapidly acting metabolites of vitamin D for short intervals prepartum may be a more effective method for the prevention of parturient paresis and, hopefully, without the toxic effects (8).

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