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OSTASIS.

The Ohio State University, Ph.D., 1971
Veterinary Science

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MICRORADIOGRAPHIC EVALUATION OF BONE AND FINE STRUCTURAL ALTERATIONS OF THYROIDAL PARAFOLLICULAR CELLS IN RESPONSE TO CHANGES IN CALCIUM HOMEOSTASIS

DISSERATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By


* * * * * *

The Ohio State University
1971

Approved by

[Signature]
Adviser
Department of Veterinary Pathology
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Major Field: Veterinary Pathology

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Studies in Pathology of Infectious Diseases. Professors Richard A. Griesemer and Robert L. Farrell

Studies in Comparative Neuropathology. Professor Adalbert Koestner

Studies in Medical Pathology. Professor Dante Scarpelli
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CHAPTER I

MICRORADIOGRAPHIC EVALUATION OF BONE FROM COWS WITH EXPERIMENTAL HYPERVITAMINOSIS D, DIET-INDUCED HYPOCALCEMIA, AND NATURALLY OCCURRING PARTURIENT PARESIS

Introduction

Increased parathyroid activity in man and animals has been associated with an increase in bone turnover, especially resorption (Riggs et al., 1965; Jowsey and Raisz, 1968). Microradiographic evidence of increased osteolysis has also been associated with prolonged hyperparathyroid activity (Jowsey 1968). The administration of vitamin D or parathyroid extract (PTE) will produce osteolysis in several experimental systems (Bélanger et al., 1963). This response of bone to parathyroid hormone has been inhibited by thyrocalcitonin (Rasmussen and Tenenhouse, 1967; Bélanger and Rasmussen, 1968).

Parturient paresis is an afebrile, hypocalcemic disorder affecting 5-10% of high producing dairy cows near parturition. This metabolic disorder is associated with an increased plasma concentration of parathyroid hormone (Mayer et al., 1969) and ultrastructural evidence of increased parathyroid activity (Capen and Young, 1967). Parathyroid hormone appears unable to mobilize mineral from the skeletal reserves at a sufficient rate and blood levels of calcium and phosphorus decrease rapidly to less than 50% of normal. Young and Capen (1968) reported the thyroid glands of cows with parturient paresis contained only 14% as much thyrocalcitonin activity as controls and many parafollicular cells
were degranulated. These findings were interpreted to suggest that a release of thyrocalcitonin by parafollicular cells in certain cows may contribute to the relative refractiveness of the skeleton to parathyroid hormone near parturition.

The structural and functional changes of bone in response to the complex hormonal alterations associated with parturition and in preparation for the demands of lactation are incompletely understood in cows. Ramberg et al. (1970) reported there was little evidence of bone resorption in cows for a period of 4 weeks before until 2 weeks after parturition. Black and Capen (1971) using hydroxyproline as an indicator of matrix metabolism observed an increase in bone catabolism from day 1 prepartum to day 3 postpartum. Urinary excretion and plasma concentration of hydroxyproline did not increase during the last month of gestation in cows that developed parturient hypocalcemia as in control cows. There are few morphologic investigations reported on bone from adult cows under either normal or pathologic conditions. The specific objectives of this investigation were: (1) to evaluate bone formation and resorptions in cows with parturient hypocalcemia compared to bone turnover in prepartal and postpartal control cows, and (2) to define the response of bone in adult cows to chronic diet-induced hypocalcemia and pharmacologic doses of vitamin D.

**Materials and Methods**

A total of 29 adult cows were used in this investigation, including 26 Jerseys, 2 Holsteins, and 1 Brown Swiss. Group 1 consisted of 10 cows with spontaneous parturient paresis and hypocalcemia. The cows included in this group developed functional disturbances pathognomonic
for parturient paresis near parturition and had a serum concentration of calcium below 5 mg/100 ml. (Table 1). Six cows developed the disease prepartum and 4 cows postpartum. Group 2 was composed of 8 control cows fed diets providing a normal intake of calcium and phosphorus. There were 3 nonpregnant and nonlactating cows, 2 cows 3 weeks prepartum, and 3 cows from 3 to 10 days postpartum. Cows in the control group did not develop functional disturbances characteristic of parturient paresis and maintained the serum concentration of calcium in the normal range (Table 1).

Group 3 consisted of 5 adult nonlactating cows that received 30 million units of vitamin D** orally in gelatin capsules daily in divided doses for 3, 5, 5, 7, and 10 days. Group 4 was composed of 6 adult nonpregnant and nonlactating Jersey cows ranging from 4 to 8 years of age. The cows received 12 pounds of a calcium-deficient diet daily with demineralized distilled water ad libitum. The experimental diet** contained 0.09% calcium and 0.17% phosphorus. Feeding of 12 pounds of diet per day provided 4.9g of calcium and 9.2g of phosphorus plus adequate nutrients to meet NRC requirements*** for dairy cows. Monosodium phosphate was added to each daily allotment to provide a total of 25 gm phosphorus and a Ca:P of 1:5. Three cows also received 30 million

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* Quadri D "400" : 400,000 USP units of vitamin D2/gm in a dry cereal carrier. Nopco Chemical Company, 60 Park Place, Newark, N.J.

** Formula supplied through the courtesy of Dr. G. P. Mayer, University of Pennsylvania, School of Veterinary Medicine, Philadelphia and prepared by Landmark Farm Bureau Cooperatives, 245 N. High St., Columbus, Ohio.

Table 1. Bone formation and resorption in cows with parturient hypocalcemia and paresis compared to control cows.
<table>
<thead>
<tr>
<th>Group</th>
<th>No. Cows</th>
<th>Bone Resorption % Total Surface (Mean ± SD)</th>
<th>Bone Formation % Total Surface (Mean ± SD)</th>
<th>Serum Concentration (mg/100 ml) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parturient Paresis</td>
<td>10</td>
<td>5.29 ± 4.84</td>
<td>7.06 ± 3.30</td>
<td>3.8 ± 0.31</td>
</tr>
<tr>
<td>(6) Prepartum (2 hr-11 da)</td>
<td></td>
<td>6.73 ± 5.25</td>
<td>8.28 ± 2.45</td>
<td>1.9 ± 0.27</td>
</tr>
<tr>
<td>(4) Postpartum (12-24 hr)</td>
<td></td>
<td>3.14 ± 1.88</td>
<td>5.38 ± 3.11</td>
<td></td>
</tr>
<tr>
<td>Control Cows</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepartum (3 wk) (2)</td>
<td></td>
<td>6.50</td>
<td>7.16</td>
<td>11.1</td>
</tr>
<tr>
<td>Postpartum (81 hr) (1)</td>
<td></td>
<td>1.69</td>
<td>1.93</td>
<td>9.1</td>
</tr>
<tr>
<td>Postpartum (7, 10 da) (2)</td>
<td></td>
<td>12.52</td>
<td>4.53</td>
<td>9.9</td>
</tr>
<tr>
<td>Nonlactating, Nonpregnant</td>
<td></td>
<td>3.13 ± 0.07</td>
<td>5.19 ± 0.54</td>
<td>10.1 ± 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.5 ± 0.38</td>
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</table>
units of vitamin D administered daily in gelatin capsules from the 20th to the 30th day of feeding the calcium-deficient diet. A section of the 10th rib from each cow was collected after feeding the experimental diet for 30 days. Serum samples were collected in all cows simultaneously with the bone specimens. The serum concentration of calcium from the cows was determined by atomic adsorption spectrophotometry (Perkin-Elmer 303). The serum phosphorus was quantitated by the method of Fiske and Subbarow (1925).

The bone specimens for microradiography were fixed in 70% alcohol (Jowsey et al., 1965) and in 10% phosphate buffered formalin for light microscopic evaluations. Bone sections fixed in alcohol were embedded in methyl methacrylate and cut at approximately 100 μ with a Gillings-Hamco thin section machine at reduced speed (500 rpm). A 4-inch stainless steel blade was used that had 310 teeth and was 0.030 inches in thickness. The sections were ground under glass to obtain a uniform thickness of 100 μ. Section thickness was critically measured by a mikrokator* with adjustable measuring pressure.

The sections were exposed in a continuously evacuated removable camera to Kodak 649 emulsion on 1 X 3 in microslides using a Philips X-ray diffraction unit with full-wave rectification. The diffraction unit was operated at 20Kv and a copper target was used since its characteristic radiation is known to be absorbed selectively by hydroxyapatite (Jowsey et al., 1965). High resolution was obtained by using a focal spot of less than 1mm in width and a target to specimen distance of 20 cm.

* Mikrokator, No. 7V2509-4, C.E.J. Gage Co. (Subsidiary of C. E. Johnsson, Sweden), 10641 Haggerty, Dearborn, Michigan 48126.
Bone remodeling was assessed directly by viewing the microradiographs under a light microscope. Bone surfaces concerned with formation and resorption were interpreted according to characteristics established by Jowsey et al. (1965). Formation and resorption surfaces were marked on photomicrographs that were enlarged 30 times. These surfaces were measured with a K and E map measurer* and expressed as a percentage of the total surface area on the section. Alterations in the size and shape of osteocytic lacunae were evaluated by microradiography and on histologic sections stained with toluidine blue. Osteoid seams were determined by viewing 100µ ground undecalcified sections of methacrylate-embedded bone from cows in groups 1 and 2 (Jowsey and Raisz, 1968).

Bone sections for histologic evaluation were decalcified for 12 to 18 hours with 15% formic acid or undecalcified and embedded in Spurr plastic (Spurr, 1969). Selected bone specimens were embedded and sectioned without decalcifications. The sections were cut at 10-15µ on a Jung microtome (Model K) and stained with either toluidine blue or hematoxylin and eosin.

Results

Microradiographic evaluation of rib sections from control cows revealed the bone was composed of numerous closely packed osteons of varying density in the cortex and trabecular bone with many inactive surfaces (Fig. 1). The majority of the osteons were closed and the central canal had a diameter of less than one-third that of the area

* K and E Map Measurer, Model 620350, Minneapolis Blue Printing Co., 612 3rd Ave., South Minneapolis, Minn. 55003.
Fig. 1. Microradiograph of the 10th rib from an adult nonlactating cow. The compact (C) bone is composed of osteons of variable radiodensity (long arrows). Several osteons (S) have sequestered bone. The interstitial (I) bone is prominent and trabecular bone has numerous inactive surfaces (short arrow). X 75.
within the cementing line. The mean perimeter of 170 osteons was 202 microns. The osteons had a line of increased density at the free border, suggesting the surface was inactive. Less than 0.5% of the osteons in cortical bone were occluded completely in control cows whereas 0.7% were occluded in cows that developed parturient paresis. These differences were not significant statistically. There were many osteons of low density near both the endosteal and periosteal surfaces. The osteocytic lacunae in the interstitial bone were frequently prominent and appeared larger than those either within osteons or in trabecular bone. Occasionally, multi-haloed osteons were observed in cortical bone of control cows which were interpreted to indicate periods of interrupted bone growth (Fig. 2). Hypermineralized (radiodense) osteons were seen infrequently (Fig. 3).

A comparison of bone from cows with parturient hypocalcemia and paresis to controls (nonpregnant-nonlactating cows and pregnant cows 3 weeks prepartum) revealed no significant difference in bone turnover (Table 1) (Fig. 4). There was little evidence of osteocytic osteolysis in either cows with parturient hypocalcemia or in control cows (Fig. 5). The 10th rib from the Holstein cows was observed to have considerably more trabecular bone than the Jersey cows (Figs. 6, 7). The cow at 81 hours postpartum had the lowest bone turnover of any control cow evaluated but had prominent osteocytic osteolysis.

Bone resorption was increased in control cows 7-10 days postpartum and there was a 3-fold difference between resorption and formation. Extensive resorption of bone was observed along trabecular surfaces but
Fig. 2. Microradiograph of compact and trabecular bone in rib from an adult nonlactating control cow. Multiple osteons of varying density (O) and interstitial lamellae with prominent osteocytic lacunae (short arrow) are present in cortical bone. Several osteons have a "halo" of increased density within the osteon (long arrow). The trabecular bone (T) has primarily inactive surfaces. X 45.
Fig. 3. Microradiograph of rib illustrating compact bone with osteons of varying density. One osteon has a central area of increased mineral density (long arrow) and a radiopaque line bordering an area of low density. Adult nonlactating control cow. X 212.
Fig. 4. Microradiograph illustrating compact (C) and trabecular (T) bone from the 10th rib of an adult Jersey cow with parturient hypocalcemia and paresis. The trabecular bone has numerous inactive surfaces (long arrow). The compact bone is composed of closed osteons of varying density with "halo" formation observed in several osteons (short arrow). The trabecular bone has areas of low density sequestrated between lamellar bone of high density (D). X 42.
Fig. 5. Microradiograph of compact bone from the 10th rib of an adult cow with parturient hypocalcemia and paresis. The osteons are of variable density and one osteon is plugged (short arrow). Note the osteon with a prominent rim of osteocytic lacunae, suggesting osteolysis. Some lacunae appear to be confluent (long arrow). X 162.
Fig. 6. Microradiograph illustrating compact (C) and trabecular (T) bone from the rib of an adult Jersey cow with parturient hypocalcemia and paresis. The outer circumferential lamellae (0) are well delineated and the adjacent interstitial lamellae (1) are prominent. The remainder of the compact bone is composed of numerous closely situated osteons of variable density (long arrows). Trabecular bone is extensive and the lamellae are of different radiodensity. X 19.
Fig. 7. Microradiograph of compact (C) and trabecular (T) bone from the rib of an adult Holstein cow with parturient hypocalcemia and paresis. Compare to Fig. 6 taken at the same magnification. Note the greater development of trabecular bone in the rib from the Holstein cow compared to the Jersey cow. Outer circumferential lamellae were not observed in the cortex and the osteons (long arrows) were not as closely arranged together. X 21.
there was only minimal osteoclastic and osteocytic resorption in the compact bone (Fig. 8).

Osteoid seams appeared as distinct yellow borders along trabecular surfaces and bordering the central canals of osteons on undecalcified bone sections embedded in methacrylate. The thickness of osteoid seams and surface area covered by osteoid were similar in bone from diseased and control cows (groups 1 and 2) (Fig. 9). There was no morphologic evidence to suggest the presence of osteomalacia in cows which developed parturient hypocalcemia and paresis.

The feeding of a calcium-deficient diet for 30 days to nonpregnant-nonlactating cows increased resorption significantly ($P<0.02$) (Table 2). There was no evidence of increased osteocytic osteolysis and the bone resorption appeared to be restricted to free trabecular surfaces. Bone formation did not differ significantly from controls. The supplementation of the calcium-deficient diet with 30 million units of vitamin D daily for the last 10 days increased bone turnover further, especially bone resorption ($P<0.05$) (Table 2). There was increased osteoclastic activity along trabecular surfaces and confluent areas of decreased radiodensity were observed surrounding the osteocytic lacunae within osteons of compact bone (Fig. 10). Bone formation remained similar to that of control cows.

The administration of pharmacologic doses of vitamin D to adult cows fed a normal diet for 3 to 10 days resulted in a marked increase in porosity of the compact bone (Table 3) (Fig. 11). There were numerous roughened and irregular resorption surfaces within the cortical bone. However, trabecular surfaces did not have an increased surface area
Fig. 8. Microradiograph of compact and trabecular bone from the 10th rib of a control cow 10 days postpartum illustrating extensive resorption along endosteal surfaces (long arrows). The inner circumferential lamellae are prominent (L). Several osteons in the compact bone have a sclerotic radiopaque surface bordering the central canal (short arrows). X 45.
Fig. 9. Undecalcified methacrylate-embedded section illustrating trabecular and central canal surfaces with a rough uneven layer of connective tissue (arrow). Note the lack of a smooth, homogenous layer of osteoid. The compact bone consists mainly of closed osteon (C). Rib from a cow with parturient hypocalcemia and paresis. Unstained, X 156.
Table 2. Effect of a calcium-deficient diet alone and with added pharmacologic doses of Vitamin D on bone turnover in adult nonlactating cows.
<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cows</th>
<th>Bone Resorption (% Total Surface) Mean ± SD</th>
<th>Bone Formation (% Total Surface) Mean ± SD</th>
<th>Serum Concentration (mg/100 ml)</th>
<th>Calcium Mean ± SE</th>
<th>Phosphorus Mean ± SE</th>
</tr>
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<tr>
<td>Nonpregnant, Nonlactating Control Cows</td>
<td>3</td>
<td>3.13 ± 0.07</td>
<td>5.19 ± 0.54</td>
<td>10.14 ± 0.34</td>
<td>6.22 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Calcium-Deficient Diet</td>
<td>3</td>
<td>5.34 ± 0.95**</td>
<td>7.16 ± 2.14</td>
<td>8.79 ± 0.08</td>
<td>9.13 ± 1.14</td>
<td></td>
</tr>
<tr>
<td>Calcium-Deficient Diet + 10 Days Vit. D</td>
<td>3</td>
<td>14.21 ± 5.91*</td>
<td>5.96 ± 2.31</td>
<td>10.74 ± 0.35</td>
<td>6.85 ± 0.85</td>
<td></td>
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</table>

** Significant: P<0.02
* Significant: P<0.05
Fig. 10. Microradiograph of cortical bone of rib from an adult cow fed a calcium-deficient diet for 30 days supplemented with vitamin D (30 million units daily) for the last 10 days. Many osteons have a prominent confluent ring of osteocytic lacunae near the cementing line (long arrows). There is a row of prominent lacunae (short arrow) at the junction of the outer circumferential (0) and interstitial lamellae (1). Many osteons have a radiopaque border adjacent to the central canal. X 145.
Table 3. Effect of daily administration of 30 million units of vitamin D on bone formation and resorption in adult nonlactating cows. ± = standard error. Values in parentheses represent the increase in serum calcium and phosphorus (mg/100 ml) above baseline levels.
<table>
<thead>
<tr>
<th>Group</th>
<th>No. Cows</th>
<th>Bone Resorption (%) Total Surface</th>
<th>Bone Formation (%) Total Surface</th>
<th>Serum Concentration (mg/100 ml)</th>
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<tr>
<td><strong>Vitamin D Administration</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>9.93</td>
<td>4.32</td>
<td>10.6 (1.5) 6.0 (0.0)</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>7.43</td>
<td>5.19</td>
<td>12.2 (1.3) 6.8 (1.3)</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>7.40</td>
<td>2.99</td>
<td>12.3 (1.4) 8.5 (2.0)</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>7.43</td>
<td>5.53</td>
<td>13.5 (3.5) 10.0 (2.2)</td>
</tr>
<tr>
<td><strong>Nonpregnant, Nonlactating Control Cows</strong></td>
<td>3</td>
<td>3.13 ± 0.07</td>
<td>5.19 ± 0.54</td>
<td>10.1 ± 0.34 6.2 ± 0.3</td>
</tr>
</tbody>
</table>
Fig. 11. Microradiograph of rib from a nonlactating adult cow receiving vitamin D (30 million units daily) for 5 days. There are numerous resorption spaces (short arrows) within the cortical lamellae. Cortical bone has increased porosity and trabecular surfaces have multiple areas of resorption (long arrow). X 18.
undergoing resorption and osteocytic osteolysis was not a prominent part of the response to vitamin D administration (Fig. 12). Although resorption was increased after vitamin D administration to cows there was no corresponding increase in bone formation during the 10 day period (Fig. 13) (Table 3).

Discussion

Bone turnover as evaluated by microradiography was low in cows which developed a syndrome of profound hypocalcemia and paresis near parturition and did not differ significantly from prepartal control cows. Investigations by Black and Capen (1971) demonstrated that bone matrix metabolism remained constant during the prepartal period in cows which subsequently developed parturient hypocalcemia and paresis at parturition. The urinary excretion and plasma concentration of hydroxyproline increased gradually for 1 month prepartum in cows which were able to maintain their serum concentration of calcium near normal through parturition and during early lactation. These findings were interpreted to suggest that during the last month of gestation the skeletal calcium reserves in diseased cows either were relatively refractory to stimuli which increased matrix catabolism or the bone received minimal stimulation.

A release of thyrocalcitonin prepartum in diseased cows may contribute to the suboptimal response of bone to the increased secretion of parathyroid hormone that occurs with the approach of parturition in cows. Previous investigations have shown that thyroid glands of cows with parturient paresis contain less thyrocalcitonin activity and many
Fig. 12. Microradiograph of 10th rib from an adult nonlactating cow receiving vitamin D (30 million units daily) for 5 days. There are multiple irregular surfaces of resorption (long arrows) in the Haversian and trabecular bone. Areas of bone resorption (short arrows) are also present along the border of the outer circumferential lamellae (0). Osteocytic lacunae are not prominent. X 42.
Fig. 12
Fig. 13. Microradiograph of trabecular bone from rib illustrating a sclerotic line of radiopacity indicative of an inactive bone surface with no evidence of formation or resorption (long arrow). Several prominent osteocytic lacunae are present in trabecular bone and are surrounded by a rim of bone of decreased density (short arrow). Adult nonlactating cow that received vitamin D (30 million units daily) for 7 days. X 175.
parafollicular cells appeared degranulated (Capen and Young, 1967; Young and Capen, 1968). Preliminary microradiographic and histologic investigations of bone from diseased cows indicated the osteoclastic response to parathyroid hormone was not impaired by osteoid accumulation on trabecular or Haversian surfaces. There was not an increased thickness of osteoid seams or a greater number of bone surfaces covered with osteoid in diseased compared to control cows. The influence of other hormones on bone metabolism and their contributions to the development of severe hypocalcemia near parturition have yet to be evaluated completely. The increased secretion of estrogens (Erb et al., 1968) and elevated plasma cortisol levels (Littledike et al., 1969) near parturition in cows would favor the development of hypocalcemia with the onset of lactational demands for calcium.

The feeding of low calcium diets or the administration of pharmacologic doses of vitamin D (Boda and Cole, 1954; Hibbs and Conrad, 1960) have been used to prevent parturient hypocalcemia and paresis in cows. The feeding of a calcium-deficient diet for 30 days significantly decreased the serum concentration of calcium to 1.87 mg/100 ml below baseline levels. A 66% increase in bone resorption along trabecular surfaces was associated with the hypocalcemia. Ultrastructurally, chief cells in the parathyroid gland were hyperactive and had arrays of endoplasmic reticulum and prominent Golgi apparatuses (C. Capen, unpublished observations). The osteocytes did not appear to respond to the parathyroid hyperactivity by increased osteolysis. Parallel studies by Young and Capen (1971) determined that the plasma thyrocalcitonin levels in these cows determined by biologic assay after oxy-
cellulose extraction remained constant even though there was a significant reduction in serum calcium. The thyroid glands contained only 55% of the thyrocalcitonin activity when compared to control cows. The lack of detectable osteocytic activity in cows receiving the calcium deficient diet may have been a reflection of selective inhibition exerted by thyrocalcitonin on osteocytes.

The addition of 30 million units of vitamin D daily to the calcium-deficient diet for the last 10 days prevented the decrease in serum calcium but did not result in hypercalcemia. Rasmussen et al. (1963) postulated that parathyroid hormone exerts its maximal effect on calcium transport in the presence of vitamin D. The enhanced osteoclastic resorption along trabecular surfaces and the profound increase in osteolysis involving the compact bone in the cows suggested that parathyroid hormone and vitamin D worked synergistically to enhance osteocytic activity under these experimental conditions.

Hypervitaminosis D has been reported to increase osteocytic osteolysis in several animal species, including the rat and chicken (Bélanger, 1963; Bélanger and Clark, 1967). Pharmacologic doses of vitamin D also have been associated with excessive resorption and failure of normal mineralization in rodents (Ham and Lewis, 1934; Storey, 1960; Carlsson, 1952). The results of the present study showed a 2-fold increase in bone resorption after administering 30 million units of vitamin D for 3 to 10 days to adult cows. There was no evidence of increased osteocytic osteolysis in osteons as was observed in cows fed the calcium-deficient diet supplemented for 10 days with vitamin D. There was an elevation in serum calcium up to 3.5 mg/100 ml above baseline levels
associated with a significant depletion in the thyroid content of thyrocalcitonin compared to control cows. Bélanger and Rasmussen (1967) have shown that experimentally induced osteolysis is inhibited by the administration of exogenous thyrocalcitonin. Although there was no evidence of increased osteocytic osteolysis on microradiographs of bone from cows in response to vitamin D, calcium appeared to be mobilized from the skeletal reserves by resorption of cortical and trabecular bone by osteoclastic activity. Conrad and Hansard (1957) have determined by studies employing $^{45}$Ca in immature Herefords a 3-fold increase in net calcium retention after vitamin D administration and increased deposition of calcium in areas of new bone formation. In this investigation the administration of vitamin D to adult cows for periods of from 3 to 10 days did not increase the percentage of bone surfaces responsible for formation, as evaluated by microradiography.

The increase in bone resorption was greater in cows receiving pharmacologic doses of vitamin D than in cows fed a calcium-deficient diet for 30 days and involved both cortical and trabecular bone surfaces. The effects of vitamin D on augmenting bone turnover, increasing the intestinal absorption of calcium, and elevating the serum calcium would appear to be the most effective single method of altering calcium homeostasis near parturition to prevent the development of profound hypocalcemia.

**Summary**

Microradiographic and histologic evaluation of cortical and trabecular bone revealed a low turnover in cows which developed a syndrome of profound hypocalcemia and paresis near parturition. However, bone
resorption and formation in diseased cows were not significantly lower than prepartal control cows. Trabecular and Haversian surfaces appeared inactive but were not covered with excessive osteoid. Lactation was associated with a 3-fold increase in resorption of trabecular bone from 7 to 10 days postpartum in control cows.

Feeding a calcium-deficient diet to cows for 30 days resulted in a significant hypocalcemia but less than a 2-fold increase in bone resorption. The addition of pharmacologic doses of vitamin D (30 million units daily) from the 20th to 30th day of feeding a calcium-deficient diet further increased bone resorption (approximately 5-fold), primarily along trabecular surfaces. The added vitamin D prevented the development of hypocalcemia but did not produce a detectable hypercalcemia. Similar levels of vitamin D administered for 3 to 10 days to cows with normal calcium and phosphorus intake resulted in hypercalcemia and prominent resorption spaces in cortical bone. Vitamin D in adult cows appeared to be more effective than a calcium-deficient diet in influencing calcium homeostasis by altering skeletal metabolism.
CHAPTER II
MICRORADIOGRAPHIC EVALUATION OF BONE AND
ULTRASTRUCTURE OF PARAFOLLICULAR CELLS IN
THE THYROID OF COWS RECEIVING PARATHYROID EXTRACT

Introduction
Calcium homeostasis in mature dairy cows undergoes severe change at parturition. A negative calcium balance and a clinical disease syndrome of progressive hypocalcemia and hypophosphatemia may develop in the puerperal period. The therapeutic use of parathyroid extract (PTE) does not alleviate the hypocalcemia in cows with parturient paresis (Hibbs et al., 1947; Jackson et al., 1962). The importance of parathyroid hormone in mobilizing calcium from the skeletal reserves in adult dairy cows has been questioned by the experimental evidence that lactating thyroparathyroidectomized cows and nonlactating parathyroidectomized cows maintain a near normal concentration of serum calcium (Stott and Smith, 1957; Mayer et al., 1966). However, the administration of 3000-5000 USP units of PTE to dry nonlactating cows has been reported to elevate the serum calcium 1 to 2 mg/100 ml (Hibbs et al., 1947; Jackson et al., 1962).

Mayer et al. (1967) determined that parathyroidectomized cows respond to PTE by increasing bone resorption. An average of 84 Mmoles/day of calcium was removed from the bone. Johnson (1966) suggested that the first surface to release calcium after parathyroid hormone stimulation is the canalicular surface and that this occurred within hours.
Bélanger et al. (1963) demonstrated the administration of PTE to dogs or chickens resulted in osteocytic osteolysis in both compact and trabecular bone. Talmage (1965) concluded from studies employing Ca\(^{45}\) that the source of calcium after PTE administration was not the trabecular surfaces but from compact bone.

The role of osteocytes in calcium homeostasis has been implied by several recent studies. However, the significance of osteocytic osteolysis in contributing to a change in the serum calcium following the administration of PTE to adult animals is not known. An elevation in serum calcium in animals receiving PTE may be secondary to increased osteoclastic or osteocytic activity and accelerated bone turnover with subsequent alterations in the size of the exchangeable calcium pool. These activities in bone are all modified by thyrocalcitonin (Bélanger and Rasmussen 1967; Rasmussen and Tenenhouse, 1967; Caniggi, Gennari, Bencini, Cesari, and Borrelo, 1970; Bordier, Hioco, Tun-Chot, 1969; Foster, 1967 and Johnston and Deiss, 1966). An increase in the serum calcium concentration has been shown to release thyrocalcitonin (Care, Cooper, Duncan, and Orimo, 1968; Lee, Deftos and Potts, 1969; Capen and Young, 1970). An evaluation of the relative importance of osteocytes and osteoclasts in PTE-induced bone resorption and the effect exerted by thyrocalcitonin will contribute to a further understanding of the ability of bone in adult cows to contribute to the maintenance of calcium homeostasis near parturition.

This study was undertaken to determine the effect of PTE on bone turnover in adult cows and to evaluate the relative contributions of osteocytic osteolysis and osteoclastic resorption to the calcium
mobilization. A third objective was to determine the effect exerted by PTE-induced hypercalcemia on the fine structure of parafollicular cells in the thyroid glands of cows.

**Materials and Methods**

Three nonpregnant, nonlactating cows ranging from 5 to 8 years of age were used in this study. Parathyroid extract* was administered intramuscularly at the level of 5000 USP units per day in three equally divided doses (8:00 a.m., 4:00 p.m., and midnight) for 5 days. Serum was collected for 3 days prior to and daily during the administration of PTE. The serum concentration of calcium and magnesium was determined by atomic absorption spectrophotometry (Perkin-Elmer model 303). Serum phosphorus was quantitated by the method of Fiske and Subbarow (1925), and alkaline phosphatase activity by the method of Bessey et al. (1946) as modified by Hausamen et al. (1967). The baseline value for calcium, phosphorus, magnesium, alkaline phosphatase, and hydroxyproline (Table 1) represents the mean of 20 determinations for each parameter prior to PTE administration.

Twenty-four hour urine samples were collected from all cows 3 days prior to and daily during the 5 days of PTE administration. Aliquots of urine (8:00 a.m., 4:00 p.m., and 12 midnight) were frozen under toluene at -20°C until assayed. Urinary total hydroxyproline levels were determined on duplicate samples by the method of Kivirikko et al. (1967). Creatinine determinations were performed as an index of urine concentration by the method of Clark (1961). Urinary hydroxyproline:creatinine

Table 1. Serum concentration of calcium, phosphorus, magnesium, alkaline phosphatase activity, and urinary hydroxyproline:creatinine (HOP:CR) in adult nonlactating cows receiving 5,000 units of parathyroid extract daily for 5 days. Mean ± standard deviation.
<table>
<thead>
<tr>
<th>PTE Administration (hrs)</th>
<th>Serum Concentration (mg/100 ml)</th>
<th>Serum Alkaline Phosphatase (IU/L)</th>
<th>Urinary HOP:CR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calcium</td>
<td>Phosphorus</td>
<td>Magnesium</td>
</tr>
<tr>
<td>Baseline</td>
<td>10.1 ± 0.35</td>
<td>6.5 ± 1.79</td>
<td>2.6 ± 0.37</td>
</tr>
<tr>
<td>8</td>
<td>9.4 ± 0.50</td>
<td>6.4 ± 0.86</td>
<td>2.6 ± 0.43</td>
</tr>
<tr>
<td>16</td>
<td>10.2 ± 0.67</td>
<td>7.4 ± 1.95</td>
<td>3.5 ± 0.88</td>
</tr>
<tr>
<td>24</td>
<td>10.2 ± 0.23</td>
<td>6.6 ± 0.31</td>
<td>2.7 ± 0.28</td>
</tr>
<tr>
<td>32</td>
<td>10.2 ± 0.72</td>
<td>5.0 ± 0.55</td>
<td>2.4 ± 0.36</td>
</tr>
<tr>
<td>40</td>
<td>10.7 ± 0.74</td>
<td>4.6 ± 0.75</td>
<td>3.0 ± 0.45</td>
</tr>
<tr>
<td>48</td>
<td>10.7 ± 0.66</td>
<td>6.3 ± 0.36</td>
<td>2.5 ± 0.17</td>
</tr>
<tr>
<td>56</td>
<td>10.8 ± 1.05</td>
<td>5.6 ± 1.30</td>
<td>2.3 ± 0.14</td>
</tr>
<tr>
<td>64</td>
<td>11.5 ± 0.42**</td>
<td>5.4 ± 1.21</td>
<td>2.6 ± 0.98</td>
</tr>
<tr>
<td>72</td>
<td>11.5 ± 0.13***</td>
<td>4.5 ± 0.61</td>
<td>2.8 ± 0.37</td>
</tr>
<tr>
<td>80</td>
<td>11.5 ± 0.49**</td>
<td>5.6 ± 1.21</td>
<td>2.5 ± 0.33</td>
</tr>
<tr>
<td>88</td>
<td>12.1 ± 1.66</td>
<td>5.5 ± 0.91</td>
<td>2.7 ± 0.60</td>
</tr>
<tr>
<td>96</td>
<td>12.4 ± 1.42</td>
<td>6.1 ± 0.71</td>
<td>2.5 ± 0.36</td>
</tr>
<tr>
<td>104</td>
<td>11.9 ± 1.08</td>
<td>6.2 ± 0.70</td>
<td>2.3 ± 0.48</td>
</tr>
<tr>
<td>112</td>
<td>12.6 ± 1.77</td>
<td>6.9 ± 1.20</td>
<td>2.6 ± 0.38</td>
</tr>
<tr>
<td>120</td>
<td>12.4 ± 1.54</td>
<td>6.0 ± 0.96</td>
<td>2.3 ± 0.37</td>
</tr>
</tbody>
</table>

*** = Statistically significant at 1% level
** = Statistically significant at 2% level
* = Statistically significant at 5% level
ratios were calculated as follows: \[
\frac{\text{hydroxyproline mg/100 ml urine}}{\text{creatinine mg/100 ml urine}}
\]

Bone biopsies were obtained from the left tenth and right eighth rib at the point of greatest curvature. Sections from 3 to 4 cm in length were removed prior to administering PTE and on the third day of PTE respectively. The bone specimens were fixed in 70% alcohol for microradiographic evaluation (Jowsey et al., 1965).

The cows were electrocuted at the termination of the study and a complete necropsy was performed. Representative tissues from all organ systems were fixed in 10% phosphate-buffered formalin. In addition, cubes (0.5 mm) of the thyroid and parathyroid glands were fixed in direct osmium tetroxide or 3% glutaraldehyde for electron microscopic evaluation (Capen et al., 1965; Hopps and Strano, 1968). The remainder of the thyroid gland was frozen at -190°C, stored at -20°C, and assayed biologically.

Representative sections of the right tenth rib were saved for microradiography at the termination of the experiment after 5 days of PTE. Bone turnover was determined on the right eighth, left and right tenth rib. After fixation the bone specimens were embedded in methyl methacrylate and sections were cut approximately 100 μ thick on a Gillings-Hamco thin-sectioning machine. The sections were polished subsequently on ground glass to obtain greater surface uniformity. Contact microradiographs were made using microslides with Kodak 649-emulsion and radiographic parameters of 20 KV, 20 MA, and a distance of 20 cm.

Bone turnover was evaluated by systematically measuring surfaces undergoing formation and resorption with a map measurer* on photomicro-

* K & E Map Measurer No. 620350, Minneapolis Blue Printing Co., 612 3rd Ave., Minneapolis, Minn. 55003.
graphs enlarged 30 times. Areas of bone formation have been reported to be smooth surfaces with borders of low density (Jowsey et al., 1965). Bone resorbing surfaces appeared as rough, irregular surfaces of high density. The remaining inactive surfaces were also measured. The percentage of total surface undergoing formation or resorption were calculated by the method of Jowsey et al. (1965). Alterations in the size and shape of lacunae in both trabecular and compact bone were evaluated from microradiographs. The bone was also evaluated by osteochrome staining of unembedded and undecalcified bone sections ground to 100 μ (Villanueva, 1967), and by polarizing light microscopy. The differences between pre- and post-PTE bone specimens was evaluated for significance by the Student t test (Freund et al., 1962).

Results

The effect of daily PTE administration on the serum concentration of calcium, phosphorus, magnesium, alkaline phosphatase activity, and urinary hydroxyproline:creatinine (HOP:CR) are summarized in Table 1. There was a gradual increase in the serum calcium concentration after day 1 which continued to the termination of the experiment at 5 days. By the third day the serum calcium was elevated significantly (<0.001) above baseline values. The serum calcium continued to rise for the following 2 days reaching a mean elevation of 2.5 mg/100 ml above baseline values. The maximal concentration of serum calcium attained was 14.6 mg/100 ml on the fifth day of PTE administration in 1 cow. The serum concentration of phosphorus and magnesium and alkaline phosphatase activity did not differ significantly compared to the baseline period. The increased urinary hydroxyproline:creatinine during the 5 days of PTE
administration suggested an increase in bone resorption. A significant (<0.001) increase was detected after 3 days of PTE.

Parafollicular cells in the thyroid glands of cows were situated in an intrafollicular or epifollicular location. They were larger and more irregular than adjacent follicular cells and had a more electron-transparent cytoplasmic matrix. An intervening rim of follicular cell cytoplasm was interposed between parafollicular cells and the luminal colloid.

The most striking alteration in parafollicular cells of cows receiving PTE for 5 days was a reduction in the number of secretion granules. Parafollicular cells in various stages of degranulation were present; however, many cells were either completely degranulated (Fig. 1) or had only a few secretory granules situated near the plasma membrane (Fig. 2). The cytoplasmic area of degranulated cells was diminished and the intercellular space between adjacent cells was widened. Organellar development was not extensive (Fig. 1). The endoplasmic reticulum was present as individual profiles and the Golgi apparatus was small.

In response to the hypercalcemia induced by 5 days of PTE, many parafollicular cells were hypertrophied and were interpreted to be in an 'actively synthesizing' stage of the secretory cycle (Figs. 2,3). They were large, irregularly polyhedral, and extended cytoplasmic projections between adjacent follicular cells. The nuclei were irregularly indented, eccentrically placed, and had peripheral condensations of chromatin. Free ribosomes were increased markedly in number and aggregated into prominent clusters (Fig. 2). Endoplasmic reticular membranes were aggregated into straight lamellar or concentric arrays and had
Fig. 1. Degranulated parafollicular cell within a follicular wall of the thyroid from a cow receiving PTE for 5 days. The cell is in close proximity to an interfollicular capillary (arrow). The cytoplasm contains individual profiles of endoplasmic reticulum (E), free ribosomes, scattered large mitochondria but no secretory granules. An intervening rim of follicular cell cytoplasm with numerous dense bodies is interposed between the parafollicular cell and luminal colloid (C). X 14,160.
Fig. 1
Fig. 2. Partially degranulated parafollicular cell within the wall of a thyroid follicle in a cow receiving PTE for 5 days. The cytoplasm contains only a few peripherally situated secretory granules (S). Microvilli of follicular cells (long arrow) extend into the luminal colloid (C). The intense aggregation of free ribosomes (short arrows) into clusters and long profiles of endoplasmic reticulum (E) suggests the cell has entered an actively synthesizing phase. X 22,770.
Fig. 3. Hypertrophied parafollicular cell situated basally within the wall of a thyroid follicle. A large concentric array of endoplasmic reticulum (E) with numerous attached ribosomes is present at one pole of the cell. Many large mitochondria occupy much of the remaining cytoplasmic area. The few secretory granules (S) present are located peripherally near the plasma membrane. Microvilli (arrow) extend from follicular cells into the luminal colloid (C). Cow receiving PTE for 5 days. X 20,000.
Fig. 3
numerous attached ribosomes (Fig. 3). The mitochondria were large and had prominent cristae. Secretion granules were few in number and were situated peripherally near the plasma membrane facing the basilar portion of the follicles. The Golgi apparatus was small and associated with few prosecretory granules.

A few parafollicular cells were interpreted to be in the "storage" phase of their secretory cycle (Fig. 4). They resembled the predominant type of parafollicular cell observed in the thyroid glands of normal cows. The cytoplasm contained numerous electron-dense secretory granules often concentrated in the pole of the cell facing an interfollicular capillary. There were scattered groups of mitochondria, individual profiles of endoplasmic reticulum, prominent Golgi apparatuses, and lipofuscin granules in the cytoplasm.

Biopsy specimens from the left tenth rib prior to the administration of PTE revealed that a greater percentage of bone surfaces were forming new bone rather than resorbing bone in adult nonlactating cows (Table 2, Fig. 5). After PTE administration there was no change in the percent of total surface involved with bone formation as indicated by surfaces of low density on microradiographs (Fig. 6). There was a 5-fold increase in bone resorption after 3 days of PTE administration (Table 2). The increase in resorption was significant (p<0.05) and was limited primarily to trabecular surfaces (Fig. 7). After 5 days of PTE there was a 3-fold increase in bone resorption compared to baseline values (Table 2). There was no evidence of osteocytic osteolysis involving either the lacunae of Haversian systems or trabecular bone after the administration of PTE for 3 and 5 days to adult cows. The lacunae in the interstitial bone of
Fig. 4. Granulated parafollicular cells containing numerous secretory granules aggregated in the pole of the cells facing an interfollicular capillary (long arrow). The extracellular perivascular space contains collagen fibers (F) and unmyelinated nerve fibers (N). The prominent Golgi apparatus (G) is associated with prosecretory granules (P) and has partially distended cisternae. Note the clusters of free ribosomes (short arrows) and individual profiles of endoplasmic reticulum (E). Occasional lipofuscin granules (L) and large mitochondria (M) are present in the cytoplasm. X 14,160.
Figure 5. Microradiograph of a transverse section of cancellous bone of the rib from an adult nonlactating cow illustrating well developed osteonal bone in the cortex and prominent trabeculae prior to administering PTE. The compact bone consists primarily of closed osteons (O) of different densities. The outer circumferential lamellae (L) are not prominent. The trabecular bone (T) consists predominantly of well-delineated, smooth inactive surfaces with little evidence of resorption. Bone biopsy prior to PTE administration. X 16.
Fig. 6. Microradiograph illustrating compact and trabecular bone from an adult nonlactating cow prior to administering PTE. The trabecular bone has smooth inactive borders of low density (arrow) adjacent to the marrow cavity. The interstitial lamellae in the compact bone have several prominent groups of osteocytic lacunae (L). X 90.
Table 2. Effect of parathyroid extract (PTE) administration on bone turnover in 3 adult nonlactating Jersey cows.
<table>
<thead>
<tr>
<th>BONE</th>
<th>FORMATION (% of total surface)</th>
<th>RESORPTION (% of total surface)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Baseline - 10th Rib (L)</td>
<td>5.46 ± 0.72</td>
<td>3.30 ± 0.05</td>
</tr>
<tr>
<td>PTE (3 Days) - 8th Rib (R)</td>
<td>5.22 ± 1.95</td>
<td>16.83* ± 7.26</td>
</tr>
<tr>
<td>PTE (5 Days) - 10th Rib (R)</td>
<td>3.89 ± 2.39</td>
<td>10.34 ± 9.32</td>
</tr>
</tbody>
</table>

* Statistically significant at 5% level
Fig. 7. Microradiograph of a rib biopsy from an adult non-lactating cow that received parathyroid extract (5,000 U.S.P./day) for 3 days. Many surfaces of trabecular bone are rough and irregular, indicative of sites of resorption (R). No pericytic osteolysis is evident in compact bone of the cortex. Many Haversian canals have radio-dense sclerotic margins (arrow), suggestive of inactive osteons. X 60.
the cortex were often prominent compared to other areas in adult non-
lactating cows (Fig. 8).

The ground sections stained with osteochrome* according to the
method of Villanueva had prominent lacunar canalicular surfaces in
osteons and in the area of interstitial bone (Fig. 9). They were more
conspicuous after administering PTE for 3 to 5 days. The lacunae were
dark brown and round with numerous fine canalicular processes extending
out from the center. The adjacent bone had a similar brown discolora-
tion. Polarizing light microscopic evaluation of these sections reveal-
ed that polarization was restricted to the area of the cementing line
and the border of the central canal.

Discussion

The results of this investigation revealed a significant elevation
in the serum calcium and urinary hydroxyproline excretion on the third
day following the administration of PTE to adult nonlactating cows.
The mobilization of calcium from skeletal reserves was associated with
a 5-fold increase in bone resorption; however, there was no evidence
of enlarged osteocytic lacunae by microradiographic evaluation of
trabecular or osteonal bone. Bélanger (1963) demonstrated osteolysis
in young dogs following the administration of similar doses of PTE
used in this study. Osteolysis could be detected after 72 hours by
both microradiography and autoradiography. The numerous Howship's
lacunae observed along trabecular and Haversian canal surfaces associated
with 2-4 mg/100 ml increase in serum calcium in the experimental cows re-
ceiving PTE suggested that osteoclasts were of more importance than

* Osteochrome, Harleco, Harlan-Leddon Co., Philadelphia, Pennsylvania
Fig. 8. Microradiograph illustrating prominent osteocytic lacunae (arrow) within dense interstitial bone of the cortex. Mineral density between the lacunae is increased. Rib from an adult nonlactating cow that received parathyroid extract for 5 days. X 29.
Fig. 9. Undecalcified ground section illustrating osteons with normal osteocytic lacunae (short arrows) and normal osteoid seams (0). Other osteons have more prominent lacunae with a well delineated canalicular network (long arrow). Note the cutting cone with a prominent border of osteoid (0). Rib from cow receiving PTE for 5 days. Osteochrome stain. X 175.
osteocytes in the mobilization of calcium.

The absence of increased osteocytic osteolysis in response to PTE may have been due to a depression of osteocytic activity by thyrocalcitonin. Belanger and Rasmussen (1970) inhibited PTE-induced osteolysis in thyroparathyroidectomized rats by the infusion of porcine thyrocalcitonin for 14 to 16 hours. Preliminary investigations in our laboratory indicate the plasma thyrocalcitonin in cows is increased (up to 251 MRC mU/L) after PTE administration compared to control (87 ± 21 MRC mU/L) cows (D. M. Young, Personal Communication). The normal serum concentration of phosphorus after PTE administration may have facilitated the action of thyrocalcitonin on inhibiting osteolysis. Hirsch (1968) has shown that phosphate augments the action of thyrocalcitonin in altering the flux of calcium from bone to the extracellular fluids. Previous investigations have shown that ruminants respond differently to exogenous PTE than monogastric animals. Although PTE stimulates hyperphosphaturia, the serum phosphorus remains normal or even elevated due to decreased endogenous fecal level loss. (Lotz, Talmage, Comar, 1954; Mayer, Ramberg and Kronfeld, 1968; Mayer, Marshak, and Kronfeld, 1966).

Parafollicular cells in the thyroid glands of cows receiving PTE were either partially or completely degranulated, suggesting a release of thyrocalcitonin. Other parafollicular cells were hypertrophied and in the 'actively synthesizing' phase of their secretory cycle. Parallel studies on this group of cows in which the thyrocalcitonin content in the thyroid was determined by biologic assay added support to the ultrastructural observation that parafollicular cells had reduced stores of hormones. The gland content of the 3 cows receiving PTE was 431 MRC mU/g
thyroid compared to 624 ± 53 MRC mU/g in control cows (D. M. Young, Personal Communication). The 30% reduction in gland content of thyrocalcitonin was attributed directly to the significant elevation in serum calcium after PTE administration. Hypercalcemia is the major stimulus for thyrocalcitonin release and small increases (5-10%) in serum calcium elevates plasma hormone levels rapidly (Arnaud et al., 1970).

Although there was no discernible increase in osteocytic osteolysis there was a 5-fold increase in bone resorption after 3 days of PTE administration. This increase in resorption was similar in magnitude to that produced by endogenous parathyroid stimulation induced by chronic dietary hypocalcemia of 30 days duration in adult cows (Rowland et al., 1971). There was an increase in urinary excretion of hydroxyproline after the administration of PTE which correlated with the enhanced bone resorption. The maximal elevation in hydroxyproline occurred after PTE had been given for 72 hours. In man and primates a similar increase in hydroxyproline excretion has been reported following the administration of PTH (Alvioli and Prockop, 1967; Johnston and Deiss, 1965), however the onset is more rapid (Keiser et al., 1964). This apparent discrepancy may be explained by variations in doses and the greater mineral density in bone of adult cows compared to man (Jowsey, 1968).

The prominent interstitial lacunae observed in adult cows have been demonstrated in normal bone from man and adult dogs (Heucks, 1966; Detenbeck and Jowsey, 1969). The osteocytes in interstitial bone are isolated from any direct blood supply and the change in lacunar size
may be secondary to necrobiosis.

Summary

The daily administration of parathyroid extract (5000 units PTE/day) to adult nonlactating cows produced a 5-fold increase above baseline values in bone resorption after 72 hours, primarily along trabecular surfaces. Osteocytic osteolysis did not appear to be increased above baseline values on microradiographs after PTE administration. The resorption was associated with increased urinary excretion of hydroxyproline and an elevation in serum calcium. Bone resorption was increased 3-fold after 5 days of PTE and serum calcium elevated 2.5 mg/100 ml above baseline levels. The serum concentration of phosphorus, magnesium, and alkaline phosphatase activity were not altered significantly by PTE.

In response to PTE-induced hypercalcemia many parafollicular cells in the thyroid were either depleted of secretion granules or the granules present were situated near the plasma membrane. Other parafollicular cells were hypertrophied and had extensive membranous arrays of endoplasmic reticulum and large aggregations of free ribosomes, suggesting a stimulation of protein synthesis in response to hypercalcemia. Correlative biologic assay studies supported the ultrastructural observation that the gland content of thyrocalcitonin in cows receiving PTE was reduced (70%) compared to control cows.


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follicular cells of cows in response to experimental hypercalcemia in-


Chapter II


