Calcium Homeostasis in the Normal and Thyroidectomized Bovine

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Summary
In three thyroidectomized mature dairy cows daily intramuscular injection of a very small dose of vitamin D (200,000 u/day/animal for six days) induced a slight but significant hypercalcemia and hyperphosphatemia which did not appear in three control cows. In three thyroidectomized steers, a physiological dose of parathormone (40 ng/min/kg for 7 hrs) or a very slight increase in daily calcium intake (40 gm/day/animal) induced a highly significant increase in plasma calcium: similar treatments had no effect on the calcemia of control steers.

Key Words: Bovine — Calcium — Calcitonin — Parathormone — Phosphorus — Vitamin D.

Introduction
Although calcitonin (CT) seems to play an important role in the etiology of Parturient Paresis in dairy cows (Barlet 1967, 1971, Care, Bates, Phillippo, Lequin, Hackeng, Barlet and Larvor 1970), the physiological significance of CT in ruminants remains obscure. The experimental evidence for protection against hypercalcemia by the thyroid gland has been based entirely on results obtained under circumstances never encountered in normal life (Inskeep and Kenny 1968). The following study was performed to investigate a possible role of calcitonin when physiological doses of vitamin D or parathormone were administered to intact and thyroidectomized animals or when these animals received a supplementation in calcium intake corresponding to conditions usually occurring in the feeding of such animals.

Animals and methods
Six, dry, ten-years-old Jersey cows, weighing 270 ± 8 kg, and six 18-months-old Jersey steers, weighing 290 ± 7 kg
were used. Animals were fed with hay (12 kg/day/animal) and grain concentrate (1 kg/day/animal) so that their daily intake for calcium and inorganic phosphorus was 65 and 40 gm respectively.

In a first study, the effect of vitamin D₃ on plasma calcium and inorganic phosphorus was tested in thyroidectomized cows. Three of them had been surgically thyroidectomized (TX) 14 days before testing. Thyroxine replacement therapy by subcutaneous injection was started two days after surgery and was given at the rate of 1 mg/day/100 kg of body weight. This was continued until the animals were sacrificed. Three TX and three sham-operated control cows were injected daily intramuscularly with 200,000 u of vitamin D₃ (Vitocetol-Proligo) for six days. Plasma samples were collected every day at 9 am by jugular puncture.

In a second experiment the effect of an infusion of parathormone (PTH) on plasma calcium and inorganic phosphorus was compared in three TX and three sham-operated control steers. TX animals were supplemented with thyroxine as previously described for cows. TX and control animals received equipotent doses of PTH (40 mg/min/kg of body weight) (Parathyroid injection, Eli Lilly) in an intravenous infusion (200 ml) over a two hour period. Serial heparinized blood samples were taken from jugular venous cannulae.

To test the possible role of CT in the protection against hypercalcemia following calcium ingestion, seven days after the second experiment the six steers (three TX and three sham-operated) received daily 40 gm of calcium (as CaCO₃, 100 gm/day/animal, added to the concentrate) for 12 days. Blood samples were collected as described for cows.

Plasma calcium and inorganic phosphorus determinations were carried out as previously reported (Barlet 1967).

**Results**

In control cows, vitamin D₃ injection (200,000 u/day for six days) induced a slight but not significant rise in plasma calcium (ΔCa = + 0.35 ± 0.18 mg/100 ml). In TX cows the rise in plasma calcium was significant 24 hr after the third injection (ΔCa = 1.22 ± 0.12 mg/100 ml, p < 0.05) and was significantly more marked (p < 0.01) and more prolonged (p < 0.01) than in control cows. In the same way, the rise in plasma inorganic phosphorus was slight and non-significant in control cows (ΔP = + 0.64 ± 0.47 mg/100 ml) and it was significantly more marked (ΔP = + 2.08 ± 0.29 mg/100 ml, p < 0.01) and more prolonged in TX cows (Fig. 1).

In controls as in TX steers, 30 min after the beginning of the infusion, PTH induced a slight but significant fall in plasma calcium (ΔCa = −0.61 ± 0.17 mg/100 ml, p < 0.05 in controls, ΔCa = −0.58 ± 0.19 mg/100 ml, p < 0.05 in TX steers). In controls, PTH then induced a significant rise in plasma calcium, maximal two hours after the beginning of the infusion (ΔCa = + 1.38 ± 0.11 mg/100 ml, p < 0.01). The same infusion also induced a significantly more marked (ΔCa = + 3.19 ± 0.17 mg/100 ml, p < 0.001) and more prolonged hypercalcemia, persisting five hours after the end of the infusion (ΔCa = + 2.29 ± 0.27 mg/100 ml, p < 0.01) in TX steers (Fig. 2). A slight and non-significant fall in plasma inorganic phosphorus was observed in TX (ΔP = −0.33 ± 0.27 mg/100 ml) as in control steers (ΔP = −0.39 ± 0.23 mg/100 ml) three hours after the beginning of the infusion; there was no significant difference between TX and controls concerning the hypophosphatemia observed.

In control steers, daily feeding supplemented with CaCO₃ (100 gm/day/animal) did not induce significant changes in plasma calcium. The same diet induced in TX steers a significant hypercalcemia 24 hours after the first ingestion (ΔCa = + 0.82 ± 0.19 mg/100 ml, p < 0.05). Twenty-four hours after the last feeding with the diet supplemented with CaCO₃, plasma calcium concentrations were 12.65 ± 0.08 and 10.30 ± 0.14 mg/100 ml for TX and control steers respectively; the difference was highly significant (p < 0.001) (Fig. 3).

**Discussion and conclusions**

Our TX animals had intact external parathyroid glands and, in the Jersey bovine, these external parathyroids comprise more than 88% of the total parathyroid weight (Mayer, Ramberg and Kronfeld 1966). TX animals were supplemented with T₄ and the daily weight gain was similar in TX (+ 0.228 ± 0.129 kg/day/animal) and in control steers (+ 0.239 ± 0.108 kg/day). Thus, neither hypoparathyroidism nor hypothyroidism appeared to have been a factor in the results obtained.

![Fig. 1](image-url) Effect of daily vitamin D₃ injections on the calcemia and phosphatemia of thyroidectomized and sham-operated control dairy cows. Values are mean ± SEM.
Calcemia $13 \text{ mg/dL}$

$\text{1 mg/dL}$

$\text{PTH}$ infusion

$140 \text{ ng/min/kg of body weight}$

Calcemia $13 \text{ mg/dL}$

$0.5$ Calcemia

$0.5$ $3.5$ $4.25$ $5.5$ $7.5$ hrs

Fig. 2 Effect of PTH infusion on the calcemia of thyroidectomized and sham-operated control steers. Values are mean $\pm$ SEM.

Fig. 3 Effect of a daily supplement in calcium intake on the calcemia of thyroidectomized and sham-operated control steers. Values are mean $\pm$ SEM.

Talmage, Neuenschwander and Krainz (1965) were first to prove that under artificial circumstances the thyroid gland can protect the rat against hypercalcemia by secreting CT. The recovery from hypercalcemia is much more rapid after a calcium salt infusion in intact rather than thyroidectomized rats and dogs (Gittes and Irvin 1966, Sturridge and Kumar 1967, Jowsey and Detenbeck 1969). When rats treated with $40,000 \text{ u}$ of vitamin D for three days were thyroparathyroidectomized, the serum calcium rose to very high values resulting in death of the animal in many cases (De Luca, Morii and Melancon 1968). In our experiment in cows, a dose of vitamin $D_3$ as low as $200,000 \text{ u/day} - 300,000 \text{ u}$ are the levels of vitamin found in $5 \text{ kg}$ of grain, a common feeding for high producing dairy cows (Hibbs and Conrad 1966) — induced a significant hypercalcemia which did not appear in controls with intact thyroid glands (Fig. 1).

The protective action of CT might also be seen when the hypercalcemia was produced in rats (Hirsch and Munson 1966) or in laying hens (Barlet and Calamy 1971) by injection of PTH. In the cow, the PTH secretion rate seems to vary between $0.5$ to $55 \text{ ng/kg/min}$ (Potts, Buckle, Sherwood, Ramberg, Mayer, Kronfeld, Deftos, Care and Aurbach 1968). In steers, a physiological dose of PTH ($40 \text{ ng/kg/min}$ for two hours) induced a significantly more marked and more prolonged hypercalcemia in TX than in control steers (Fig. 2). Thus in the bovine, CT might counteract hypercalcemia induced by high levels of endogenous PTH. PTH infusions had no significant effect on the phosphatemia of our animals: phosphorus homeostasis during parathyroid extract administration to cows appears to be preserved by a reduction in fecal phosphorus output and by accelerated turnover of bone phosphorus (Mayer, Ramberg and Kronfeld 1968).

Several years ago Copp and Cameron (1961) demonstrated that a slight but significant hypocalcemia occurred quickly after injection of parathyroid extract in dogs. A similar hypocalcemia can be observed in steers $30 \text{ min}$ after beginning the infusion of PTH (Fig. 2). This might be due to the initial shift of calcium into bone as recently shown in rats injected with PTH (Parsons and Robinson 1971).

In thyroparathyroidectomized, milk-fed rats, plasma calcium is significantly higher than in intact milk-fed controls (Lederer, Stein and Arnould 1969). In the same way, a significant increase in serum calcium persists two hours after an oral calcium load ($1-8$
mg of Ca/rat) in thyroidectomized rats, as compared with an inconsequential effect in sham-operated controls (Gray and Munson 1969, Munson and Gray 1970). In steers used in the above experiment, an increase in calcium intake as low as 40 gm/day (i.e. 2 kg of lucerne hay/day) induced a highly significant hypercalcemia in TX animals, which did not appear in the control animals (Fig. 3). Thus, in the bovine as in the rat, CT seems able to prevent a rise in plasma calcium induced by the diet; this might explain why an impressive rise in plasma calcium is never observed with common bovine rations high in calcium. When ingested calcium is absorbed from the intestinal tract in intact animals, it might stimulate CT secretion by a slight rise in plasma calcium concentration. In young growing wethers weighing 30 kg, when calcium intake increases from 400 to 580 mg/day, the resorption rate of calcium from bone decreases from 130 to 90 mg/day (Braithwaite and Riazzudin 1971) and CT inhibits bone catabolism (Milhaud, Perault and Moukhtar 1965). In the intestinal tract, ingested calcium might also influence the secretion of gastrin, a polypeptide hormone which appears now as a possible CT-releasing factor (Cooper, Schwesinger, Mahgoub and Ontjes 1971).

We conclude that in the growing steer, as in the mature dairy cow, a physiological role of calcitonin is to protect against hypercalcemia induced by a rise in plasma PTH levels or by enhanced absorption of calcium from the gastro-intestinal tract. The calcitonin role may explain the lack of effect on plasma calcium concentration in the ruminant animal having wide variation in the calcium content of its ration.

Acknowledgments: The author wishes to thank Professor A.D. Care for advice and stimulating discussions. Parathormone and vitamin D3 used in this experiment were kindly supplied by Eli Lilly France and Prolipe. The technical assistance rendered by A. Bellanger, P. Chagnaud and J. Overwater is gratefully acknowledged.

References


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