

Inhibition of Bone Resorption: An Important Mechanism in the Pathogenesis of Parturient Hypocalcemia

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Introduction

Investigations into the pathogenesis of parturient paresis have demonstrated that parathyroid insufficiency is not a major etiologic factor (1,2). However, a common finding in several studies on naturally-occurring parturient paresis has been a decreased rate of bone resorption (3,4). Some investigations have correlated parturient hypocalcemia and hypophosphatemia with inappropriately high circulating levels of calcitonin (5). Bioassay for calcitonin activity in the thyroid glands of diseased cows demonstrated a significantly lower activity compared to normal parturient cows (6). The inappropriate secretion of calcitonin prepartum may be one factor that contributes to the inability of increased levels of parathyroid hormone to mobilize calcium rapidly from skeletal reserves and maintain adequate blood calcium levels near parturition in certain cows (7).

The composition of the prepartal diet is an important factor in the cause and prevention of parturient paresis (8). Low calcium prepartal diets have been effective in preventing the occurrence of parturient paresis in high incidence herds (9). By comparison, high calcium diets fed prepartum have resulted in a higher incidence of parturient hypocalcemia (10). The ingestion of high levels of calcium prepartum increased plasma calcium levels (11), but resulted in lower calcium levels at parturition in association with decreased secretory activity of chief cells in the parathyroid glands (12).

A syndrome biochemically and clinically resembling naturally-occurring parturient paresis has been produced experimentally in cows by the prepartal administration of diphosphonates (14). These compounds are synthetic analogues of pyrophosphate and

are proven inhibitors of bone resorption (13). When cows with no known history of parturient paresis were fed a low calcium diet and administered dichloromethane diphosphonate (CL_2MDP), they consistently developed hypocalcemia and hypophosphatemia postpartum with muscular weakness, incoordination and eventually sternal or lateral recumbency (15). These studies strongly suggest the importance of the skeleton in the maintenance of calcium homeostasis at parturition in dairy cows.

The objectives of this report are: first, to summarize the effects on calcium homeostasis of feeding high, normal, and low calcium diets prepartum as well as the effects of administering a chemical inhibitor of bone resorption (dichloromethane diphosphonate); and second, to relate these results to the pathogenesis of parturient paresis.

High Calcium Diet

Investigations in our laboratory demonstrated that cows fed a high calcium (150 gm of calcium, 25 gm of phosphorus per day) prepartal diet for 60 days had higher levels of serum calcium prepartum than cows fed a normal calcium diet but were less able to maintain serum calcium at normal levels near parturition (Table 1). Serum phosphorus decreased similarly during the first 48 hours postpartum. Ultrastructurally, chief cells in the parathyroid glands of cows fed the high calcium diet were predominantly in the inactive phase of the secretory cycle or atrophic (12). Plasma parathyroid hormone levels were consistently lower prepartum in cows fed the high calcium diet and declined at 48 hours postpartum in comparison to values for cows fed a prepartal diet with the required amounts of calcium and phosphorus. The results of these investigations suggested that the long-term feeding of a high calcium prepartal diet suppressed the ability of chief cells to respond rapidly to a hypocalcemic challenge associated with parturition and initiation of lactation by increased synthesis and secretion of parathyroid hormone. Urinary hydroxyproline excretion, an indicator of bone matrix catabolism, was lower in cows fed a high calcium diet than in cows fed a low calcium diet at one day postpartum (Table 1). Bone resorptive surfaces, determined by microradiography, also were fewer compared to values for cows fed either a low or normal calcium diet (Table 2). Similar findings of decreased urinary hydroxyproline excretion (3,16) and fewer resorptive surfaces (4) have been reported in cows with naturally occurring parturient paresis compared to normal parturient cows. Fine structural evaluation demonstrated that thyroid C-cells of cows fed a high

Table 1. Serum calcium and phosphorus, plasma parathyroid hormone (iPTH), and urinary cyclic adenosine mono-phosphate (cAMP) and hydroxyproline (HOP) in cows fed high, control, or low calcium diets and in cows administered a chemical inhibitor of bone resorption (CL₂MDP).

	High Calcium (150g Ca, 25g P)	Normal Calcium (25g Ca, 25g P)	Low Calcium (9.5g Ca, 25g P)	Low Calcium + CL ₂ MDP (9.5g Ca, 25g P) (4 mg/kg/day)
Serum Calcium (%)	79	89	89	48
Serum Phosphorus* (%)	84	92	101	47
Plasma iPTH* (%)	110	121	111	326
Urinary HOP (%)	383	345	553	241
Urinary cAMP*%	271	211	132	346

*Values at 1 day postpartum expressed as percent of baseline levels at from 60 to 90 days prepartum.

Table 2. Intestinal calcium-binding protein (CaBP), thyroid calcitonin activity, and bone resorption 1 day postpartum in cows fed high, control or low calcium diets and in cows administered a chemical inhibitor of bone resorption (CL₂MDP).

	High Calcium (150g Ca, 25g P)	Normal Calcium (25g Ca, 25g P)	Low Calcium (9.5g Ca, 25g P)	Low Calcium + CL ₂ MDP (9.5g Ca, 25g P) (4 mg/kg/day)
Intestinal CaBP (sp. activity) 45 Ca/mg protein)	0.27**	0.32**	0.61	0.44
Calcitonin Activity (MRC mU/gm)	431 **	481 **	655	468
Bone Resorption (% Total Surface)	3.53**	4.90**	9.40	1.80*

*P < 0.01 compared to values for cows fed the low calcium diet.

**P < 0.05 compared to values for cows fed the low calcium diet.

calcium diet were partially degranulated and predominantly in the actively synthesizing phase of the secretory cycle (17). Levels of thyroid calcitonin activity, determined by bioassay, were lower in cows fed the high calcium diet than for cows fed either a normal or low calcium diet (Table 2). Calcitonin has been shown to temporarily inhibit parathyroid hormone-stimulated bone resorption (18). These observations are consistent with the concept that an inappropriate secretion of calcitonin (5,19) contributes to the inhibition of bone resorption that occurs near parturition in cows that develop parturient paresis.

Immediately available calcium reserves were less at 10 days prepartum in cows fed the high calcium diet, as indicated by the slower rate of return to normal of serum calcium following ethylenediaminetetraacetic acid (EDTA)-induced hypocalcemia (Figure 1). Urinary hydroxyproline excretion was decreased similarly compared to controls during the EDTA infusion (12). These findings suggest that cows with a high calcium intake not only had less rapidly mobilizable calcium reserves but the reduction was due to an impaired catabolism of bone matrix and osteoclastic resorption of bone.

In cows fed the high calcium diet the *in vitro* calcium⁴⁵ uptake and concentration of calcium in the duodenal mucosa was higher than for cows fed a normal calcium diet (17). The greater calcium uptake occurred despite a decreased amount of calcium-

binding protein (Table 2), which is suggestive of less efficient vitamin D-facilitated active transport of calcium by the intestine (20). These findings in association with observations of impaired resorption of bone indicate that cows fed a high calcium diet prepartum are largely reliant on intestinal uptake of calcium by passive and active transport mechanisms for maintaining calcium homeostasis near parturition. This greater reliance on intestinal absorption probably is a significant factor in the more frequent development of profound hypocalcemia near the initiation of the lactational calcium drain in cows fed high calcium prepartal diets. These cows would be more susceptible to the decreased calcium available for absorption which results from the anorexia and gastrointestinal stasis often associated with parturient hypocalcemia.

Normal Calcium Diet

The feeding of a prepartal diet with the required amounts of calcium and phosphorus (25 gm of calcium, 25 gm of phosphorus per day) maintained the serum calcium and phosphorus postpartum at approximately 90 percent of baseline levels at 60 days prepartum (Table 1). Chief cells in the parathyroid glands of cows fed the normal calcium diet had fine structural features consistent with active hormonal synthesis (13). At one day postpartum, the plasma levels of parathyroid hormone were greater than in cows fed the high calcium diet (Table 1).

The results of an EDTA infusion given 10 days prior to parturition indicated that cows fed a prepartal diet with the required amount of calcium had more rapidly mobilizable calcium reserves than cows fed either a low or high calcium prepartal diet (Figure 1). The urinary excretion of hydroxyproline was significantly higher at selected intervals when compared to values from cows fed a high calcium prepartal diet (12). These findings suggest that the immediately available calcium reserves of cows fed the normal calcium diet are directly related to active resorption of bone.

In our laboratory, we have demonstrated that duodenal calcium-binding protein and presumably vitamin D-facilitated transport of calcium (20,21) in cows fed a normal calcium diet was significantly less than for cows fed a low calcium diet (Table 2). Resorptive surfaces of bone were significantly higher than for cows on the high calcium intake but less than cows fed the low calcium diet (Table 2). These observations suggest that calcium homeostasis in cows fed a normal calcium diet is more dependent on bone matrix catabolism and resorption than on the active transport of calcium by the intestine. The observation that cows fed a normal calcium diet are more reliant on rapid resorption of bone for the maintenance of calcium homeostasis may explain the preventative effects that a prepartal diet with the required amount of calcium has against the development of parturient hypocalcemia.

Low Calcium Diet

Recent studies demonstrated that the feeding of a low calcium diet prior to parturition was effective in decreasing the incidence of parturient paresis in highly susceptible cows (9). Investigations in our laboratory and elsewhere (22) have shown that the rate of return of serum calcium was more rapid following a 4-hour EDTA infusion given 10 days prepartum in cows fed a low calcium (9.5 gm of calcium, 25 gm of phosphorus per day) diet for 60 days compared to cows fed a high calcium diet (Figure 1). Levels of parathyroid hormone were elevated in cows fed the low calcium diet during the EDTA infusion (23). At selected intervals for 20 hours post-EDTA infusion, values of urinary cyclic adenosine monophosphate (cAMP) and hydroxyproline were significantly elevated for cows fed the low calcium diet (23). These results indicated that the secretion of parathyroid hormone increased resorption of bone and stimulated target cells in the kidney (24). A previous study demonstrated that urinary cAMP excretion in cows which developed parturient paresis was consistently lower prepartum, at parturition and postpartum compared to cows which maintained near normal blood calcium levels at parturition (5).

Serum calcium and phosphorus remained within normal limits while plasma parathyroid hormone levels increased with the approach of parturition in cows fed a low calcium diet (Table 1). Ultrastructural evaluation of parathyroid glands at one day postpartum revealed that chief cells were predominantly in

the actively synthesizing phase of the secretory cycle. Thyroid C-cells of cows fed a low calcium diet appeared to be predominantly in the storage phase of the secretory cycle. The cytoplasmic area of C-cells was packed with numerous calcitonin-containing secretory granules. These ultrastructural findings were consistent with the results of calcitonin bioassay performed at one-day postpartum in which thyroids from cows fed the low calcium diet contained significantly greater calcitonin activity than cows fed either high or normal calcium diets (Table 2).

Urinary hydroxyproline excretion (Table 1) and the percent of bone surfaces undergoing resorption (Table 2) for cows fed the low calcium diet were significantly higher than values for cows fed either high or normal calcium diets at one-day postpartum. The specific activity of intestinal calcium-binding protein also was significantly higher for cows fed a low calcium diet (Table 2) which suggests that vitamin D-dependent transport of calcium in the intestine was more efficient in cows fed the low calcium diet.

The results of these studies suggest that calcium homeostasis in cows fed a low calcium diet is more under the fine control of parathyroid hormone-mediated bone resorption with the approach of parturition than in cows fed a high calcium diet. While cows fed the low calcium diet have a more efficient intestinal absorption of calcium, cows fed high calcium diets appear more reliant on high intestinal uptake of calcium

SERUM CALCIUM RESPONSE DURING AN EDTA INFUSION ADMINISTERED TO COWS 10 DAYS PREPARTUM

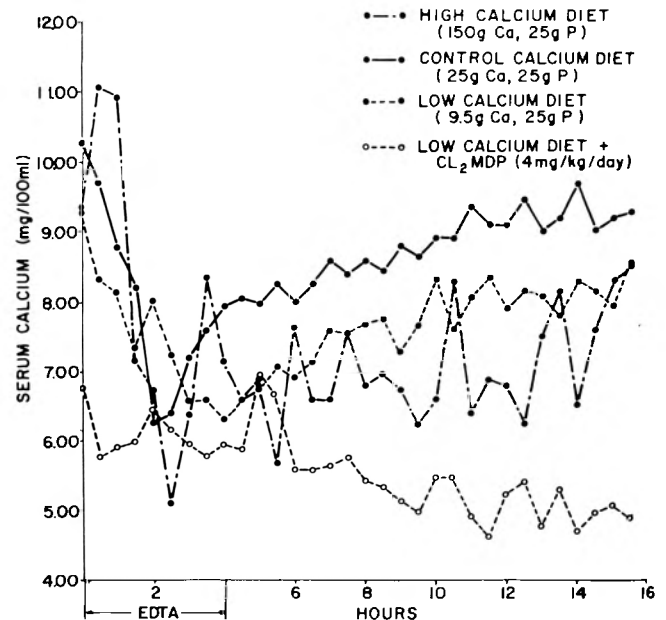


Figure 1. Immediately available calcium reserves determined by the response of serum calcium to a 4-hour EDTA infusion given approximately 60 days after feeding high, normal, and low calcium diets or administration of a chemical inhibitor of bone resorption (CL₂MDP). Values for each interval represent mean serum calcium (mg/100 ml).

rather than bone resorption as a means to maintain calcium homeostasis. Cows fed low calcium diets would be less susceptible to the influence of decreased calcium absorption and flow into the extracellular calcium pool resulting from anorexia and intestinal stasis associated with parturient hypocalcemia. Previous investigations have demonstrated that cows which spontaneously develop parturient paresis have a low percent of bone surfaces undergoing resorption (4). The ability of an expanded population of active bone resorbing cells to rapidly mobilize skeletal calcium in response to an initiation of the lactational calcium drain may be the significant mechanism by which a low calcium diet can prevent the development of parturient paresis.

Chemical Inhibition of Bone Resorption by Diphosphonates

The subcutaneous administration of dichloromethane diphosphonate (CL₂MDP) (4 mg/kg/day) for 60 days caused markedly lower values of serum calcium and phosphorus in cows near parturition, despite the prepartal feeding of a low calcium (9.5 gm of calcium, 25.0 gm of phosphorus per day) diet (Table 1). Bone resorption with the approach of parturition was significantly reduced as indicated by lower levels of urinary hydroxyproline (Table 1) and a decreased percent of resorptive surfaces on endosteal bone (Table 2). Ultrastructurally, chief cells of the parathyroid glands were predominantly degranulated and in the actively synthesizing phase of the secretory cycle. Levels of plasma parathyroid hormone prepartum and near parturition were as high or higher than values for cows fed the low calcium diet (Table 1). It would appear that the secretion of parathyroid hormone in the CL₂MDP-treated cows was increased in response to the severe parturient hypocalcemia. However, despite the parathyroid hormone stimulation, as indicated by elevated levels of urinary cAMP (Table 1), the bone appeared to be refractive to matrix catabolism and resorption. Previous investigations have shown that CL₂MDP may inhibit bone resorption by coating and protecting hydroxyapatite crystals from parathyroid hormone-induced osteoclasts (13,25).

Thyroid C-cells were predominantly in the storage phase of the secretory cycle in cows administered CL₂MDP and fed a low calcium prepartal diet. Bioassay of thyroid glands demonstrated a mean calcitonin activity of 468 MRC mU/gm for CL₂MDP-treated cows compared to 655 MRC mU/gm for the cows only fed the low calcium diet (Table 2).

Following an intravenous EDTA infusion given 10 days prepartum, CL₂MDP-treated cows developed a severe and prolonged hypocalcemia despite elevated levels of parathyroid hormone (Figure 1) (15). Urinary hydroxyproline excretion was low and remained relatively unchanged during the 24 hours of the EDTA study (15). These observations indicate that the rapid mobilization of calcium reserves in cows administered CL₂MDP prepartum was impaired because of diminished resorption of bone even though there was

an increased secretion of parathyroid hormone in response to severe hypocalcemia induced by parturition or EDTA. Black and Capen (3) have reported that the excretion of urinary hydroxyproline did not increase significantly during the last month of gestation in cows that developed parturient paresis.

The uptake of calcium⁴⁵ by the duodenal mucosa was greater for CL₂MDP-treated cows compared to cows fed either the normal or low calcium diets. Calcium-binding protein in the duodenum was higher in the diphosphonate-administered cows than for cows fed a normal calcium diet (Table 2). These results suggest that the CL₂MDP-treated cow which developed parturient hypocalcemia were similar to cows fed a high calcium diet in that they were more dependent upon calcium absorption from the gastrointestinal tract than upon skeletal calcium mobilization for the maintenance of calcium homeostasis. The results of studies utilizing diphosphonates in cows (13,15) demonstrated conclusively that chemical inhibition of bone resorption resulted experimentally in the development of a syndrome that biochemically and clinically was indistinguishable from naturally-occurring parturient paresis.

In conclusion, factors which alter the size of the readily available calcium pool (e.g., calcium content of prepartal diet and diphosphonates) significantly influenced the ability of the parturient cow to maintain normocalcemia with the initiation of lactation. Low and normal calcium prepartal diets increase the mobilizable calcium pool principally through an increase in bone resorption and thereby are effective in reducing the incidence of the parturient hypocalcemia. High calcium prepartal diets have the opposite effect by diminishing parathyroid hormone-mediated bone resorption and placing an overreliance on intestinal calcium transport.

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Report on Joint Workshop on Health and Production of Australian and Local Cattle in Southeast Asia

Organisational Aspects

The First Joint Conference and Workshop on Health and Production of Australian and Local Cattle in Southeast Asia was held at the Regent Hotel in Kuala Lumpur, Malaysia, from 16-20 May, 1977. It was organised jointly by the Association of Veterinary Surgeons Malaysia and the Australian Association of Cattle Veterinarians, a special interest group of the Australian Veterinary Association.

The organising committee in Kuala Lumpur comprised: conference chairman, Dr. Ahmad Mustaffa b.Hj. Babjee; secretary, Dr. Barbara Vanselow; treasurer, Dr. Len C. Lloyd; members—Dr. Anwar Hassan, Dr. Abdul Rahman b.Hj. Mohd Salleh, Dr. Abdul Latif Ibrahim, Dr. Roshi Mai Lam and Dr. Yap Teow Chong; and conference co-ordinators—Dr. G.I. Alexander and Dr. Len C. Lloyd.

The Genesis of the Workshop

A background paper prepared for the workshop by Dr. S. Thuraisingham, Director-General of Veterinary Services, summarised the events and policies in Malaysia which made the workshop timely and pertinent.

The Malaysian government has endeavoured to expand the production of animal proteins over the last twenty years. Initially, emphasis was placed upon the swine and poultry sectors with the result that the country is now self-sufficient in eggs, poultry meat and pork. However, only about 5% of the milk and about 80% of the beef consumed are produced locally. In addition, consumption of these two products is low.

The increasing emphasis being placed on milk and meat production is part of the National Livestock Policy which aims at self-sufficiency or near self-sufficiency by 1990. The New Economic Policy of the Malaysian government is promoting the development of the livestock production as one of its major components.

Livestock Programmes

Majuternak has developed a number of farms on which it has established both beef and dairy cattle herds. The original emphasis was placed upon obtaining cattle from Australia which were both heat and tick tolerant. It was also proposed to obtain as many dairy animals of this type as possible. The small pool of dairy animals of this type in Australia has led to a greater proportion of beef animals than originally intended and to interest in the use of pure European-type dairy cattle such as the Friesian under modified environmental conditions. The rationale of this approach is that the economics of production of Friesian cattle under stall-fed con-

ditions might be more attractive than that of heat- and tick-tolerant cattle under grazing conditions.

The Veterinary Department has negotiated for the supply of calves from Sahiwal by Friesian or A. I. S. matings from both New Zealand and Australia with a view to dissemination to village farmers. Pilot studies have indicated that the use of milking cows with quite modest levels of milk production can provide a markedly increased income for village farmers and result in an increased standard of living. A support structure of milk collection centres, etc., are being built up to assist in the collection and processing of this milk.

Some difficulties have arisen in the importation of cattle from Australia, particularly with regard to outbreaks of tick fever and tick control measures.

It was against this background that the workshop was organised to allow a group of Australians experienced in tropical cattle production and tick and tick fever problems to discuss at first hand the needs and problems in Malaysia.

The Workshop

The workshop centred around: (a) requirements for importation of livestock into Malaysia; (b) performance and breeding programmes of European and crossbred cattle in Malaysia and Australia; (c) tick control and tick fever; and (d) other diseases in Malaysia likely to affect cattle production.

Recommendation

In the final plenary session, the workshop recommended that a further workshop be held as soon as possible. It was also recommended that it include veterinarians from other Southeast Asian countries and that it be held in conjunction with the Australian Veterinary Association's Annual Conference in Townsville in 1979.

Acknowledgements

The three members of the organising committee notable to the Australian participants for their efforts were Dr. Ahmad Mustaffa b.Hj. Babjee, Dr. Yap Teow Chong and Dr. Len C. Lloyd.

(This report was prepared by Dr. Glenn R. Murray, Hon. Secretary, Australian Association of Cattle Veterinarians.)