# The Use of 1,25-Dihydroxycholecalciferol in the Prevention of Parturient Hypocalcemia in Dairy Cows

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## Introduction

Parturient hypocalcemia ("milk fever") is a costly disease to the dairy industry. The overall incidence of the disease has been estimated to be 5% (12) but it may approach 100% in some herds. Financial loss from the disease occurs as a result of death of affected cows, cost of treatment, and from complications arising from regurgitation of ingesta into the lungs, bloat, musculo-skeletal injury, and mastitis. If untreated by organic calcium solutions, at least 60% of cows die (9).

Effective prophylaxis offers the best hope to curtail the financial losses from parturient hypocalcemia. Currently, the practical methods of prevention include dietary control of calcium and phosphorus intake during the dry period (2,6), and the prepartal administration of vitamin D (7,8,10).

Although the herd incidence may be greatly reduced by management of dietary calcium intake prepartum, certain cows still develop parturient hypocalcemia at succeeding parturitions. The use of vitamin D has been studied extensively in an attempt to aid in the maintenance of the blood calcium and phosphorus levels in these particularly susceptible cows. The major biologic actions of vitamin D are to increase absorption of calcium and phosphorus from the intestine and to potentiate the effects of parathyroid hormone on stimulating bone resorption. These biologic actions of vitamin D are beneficial in increasing the blood calcium concentration near parturition and preventing the development of parturient hypocalcemia.

Hibbs and Pounden (8) reported that 20-30 million units of vitamin $D_2$ fed daily for at least three but no more than seven days prior to parturition prevented approximately 80% of parturient hypocalcemia. The major disadvantage of vitamin D (either cholecalciferol or irradiated ergosterol) in the prevention of parturient paresis is that the exact date of parturition must be accurately predicted. Unfortunately, some cows deliver before the minimum of three days

of vitamin D feeding are completed, whereas parturition in other cows occurs after the maximum seven-day regimen. Protection is sustained for only one day after the cessation of vitamin D administration. If vitamin D is discontinued and the cow has not delivered by the next day, the incidence of parturient paresis may be greater than if vitamin D had not been given (7). In 164 parturitions reported by Hibbs and Conrad (7), 10 cows delivered too early (i.e., before three days of vitamin D) and 41 cows delivered too late (i.e., after seven days of vitamin D) to be included in the treatment schedule.

Treatment beyond 10 days may result in widespread mineralization of soft tissues (3). This potentially serious toxicity makes the administration of pharmacologic doses of vitamin D to pregnant cows beyond 10 days hazardous. Because of these disadvantages, vitamin D has never gained widespread clinical usage in the prevention of parturient paresis.

Recent studies have demonstrated vitamin D undergoes a two-step process of metabolic activation prior to exerting its physiologic action on target cells primarily in intestine and bone (for reviews see 4,13,17). Vitamin $D_3$ (cholecalciferol) is initially hydroxylated in the liver to form 25 hydroxycholecalciferol (25-OH $D_3$). Another hydroxyl group is added to the molecule in the kidney to form 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$). This latter compound has been reported to be the principal final active metabolite or hormonal form of vitamin D.

Recently, some of the more active metabolites of vitamin D have been investigated for use in the treatment and prevention of parturient hypocalcemia in dairy cattle. 25-hydroxycholecalciferol (25-OH $D_3$) was reported to prevent parturient hypocalcemia at the dose level of 4-8 mg injected intramuscularly (15). The overall incidence was reduced from 29% in controls to 16% in treated cows at the 4 mg dosage and from 52% in controls to 19% in treated cows at the 8 mg dose level. No clinical evidence of hypervitaminosis D was detected (15). 25-OH $D_3$ did not

reduce the relapse rate when administered with calcium borogluconate as a treatment for clinical cases of parturient hypocalcemia and paresis (14). Frank, et al. (5), reported that 25-OH D3 significantly reduced the incidence of parturient hypocalcemia when given intramuscularly at least three days prepartum and repeated weekly to a maximum of three doses. The optimal dosage appeared to be 4 mg (5).

1α-hydroxycholecalciferol (1α-OH D3), a synthetic analogue of 1,25-(OH)2D3 that requires hydroxylation only in the liver, also has been studied in dairy cows for the prevention of parturient hypocalcemia. One injection of 1.0 - 1.5 μg/kg body weight given intramuscularly or intravenously at the onset of parturition was found to diminish the fall of calcium and phosphorus (1). Sachs, et al. (16), reported that 350 μg of 1α-OH D3 given to 23 parturient hypocalcemia-prone cows prevented the development of the disease if injected within 72 to 24 hours prior to parturition. Cows were injected 1-3 times at 48-hour intervals prior to parturition.

It was hypothesized that 1,25-(OH)2D3, of the vitamin D metabolites, should offer the best hope for successfully preventing the development of parturient hypocalcemia and eliminating certain disadvantages of the parent vitamin D compound. Since it is the final active metabolite of vitamin D and no further metabolism is required prior to acting on target cells in bone and intestine, the onset of action is more immediate and the potency greater than with other metabolites. Therefore, the objectives of this study were to determine the dose response of 1,25-(OH)2D3 in dairy cows and to evaluate several dose schedules to more precisely regulate the blood calcium and phosphorus near parturition in pregnant cows highly predisposed to develop parturient paresis.

## Dose Response of Cows to 1,25-Dihydroxycholecalciferol (1,25-(OH)2D3) and 1 Alpha-Hydroxycholecalciferol (1α-OH Vit. D3)

The objective of this part of the investigation was to determine the effects of varying levels of 1,25-(OH)2D3 administered both as intravenous and intramuscular doses on serum and urine electrolytes in adult non-lactating dairy cows, compared to the same cows administered identical doses of 1α-OH Vit. D3 intramuscularly, and to placebo-injected controls.

Five non-lactating, clinically normal dairy cows were used in this study. Intravenous catheters were placed in the jugular vein to inject the steroid and collect blood samples at two-hour intervals for 24 hours and three-hour intervals for the next 12 hours. Following administration of the high dose of steroid (600 μg), blood and urine were collected at daily intervals for an additional four days to determine the persistence of the effect. Urine samples were collected using indwelling catheters. The cows were administered 30, 90, 270, and 600 μg of 1,25-(OH)2D3 in-

travenously in a vehicle (10 ml) of 95% ethanol and 1,2-propanediol (1:1). Blood and urine from cows administered 1,25-(OH)2D3 and 1α-OH Vit. D3 intramuscularly in an oil vehicle were collected at six-hour intervals for three days and at 12-hour intervals for two additional days. Each dose of 1,25-(OH)2D3 and 1α-OH Vit. D3 was separated by an interval of at least 10 to 14 days. A similar volume of vehicle alone was injected to establish baseline values for the five cows. The cows were fed a diet of grain and leguminous hay that supplied the National Research Council's recommendations for calcium and phosphorus of non-pregnant cows. Serum calcium was determined by atomic absorption spectrophotometry, serum phosphorus coloimetrically, and urinary hydroxyproline by the method of Kivirikko, et al. (11), and expressed as a ratio to creatinine.

All four levels of 1,25-(OH)2D3 given intravenously significantly increased serum calcium above baseline values by 22 hours post-injection. Mean serum calcium in cows administered 600 μg 1,25-(OH)2D3 increased progressively from 8.44 + .10 mg/dl to 9.38 ± .14 mg/dl (P<.05) at 10 hours, to 10.9 ± .33 mg/dl at 18 hours (P<.01), up to 11.5 ± .32 mg/dl at 27 hours (P<.001) (Figure 1). The earliest significant (P<.05) increase occurred at 8 hours in cows administered 600 μg of 1,25-(OH)2D3 with a peak increase of 3.5 mg/dl at 27 hours post-injection. Serum phosphorus was increased significantly above baseline values by all four dose levels of 1,25-(OH)2D3 given intravenously by 10 hours (Figure 2). Mean serum phosphorus in cows administered 600 μg 1,25-(OH)2D3 increased progressively from 4.3 ± .27 mg/dl to 6.37 ± 0.29 mg/dl at 22 hours post-injection and persisted above the baseline levels throughout the 120 hours.

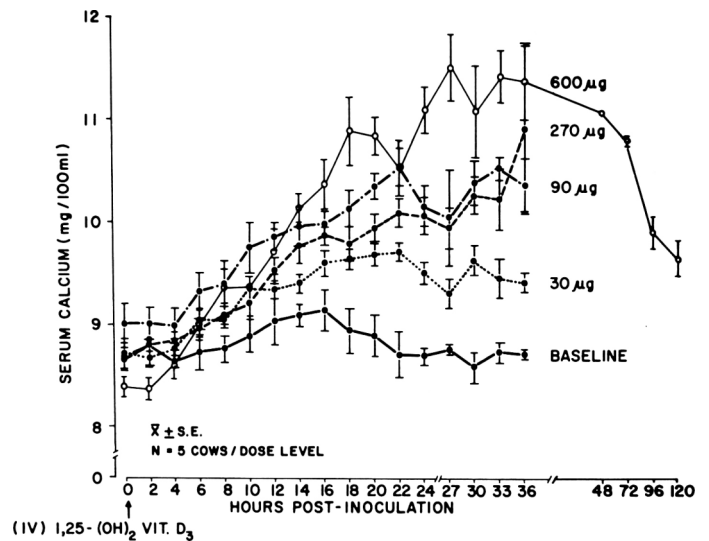**EFFECT OF 1,25- DIHYDROXYCHOLECALCIFEROL ON SERUM CALCIUM**



Figure 1. Changes in serum calcium for 36 hours following administration of 30, 90, 270, and 600 μg 1,25-(OH)2D3 intravenously to cows compared to baseline values. Cows administered 600 μg of 1,25-(OH)2D3 were followed an additional four days to determine the duration of the hypercalcemic effect. Five cows were used per dose level of 1,25-(OH)2D3.

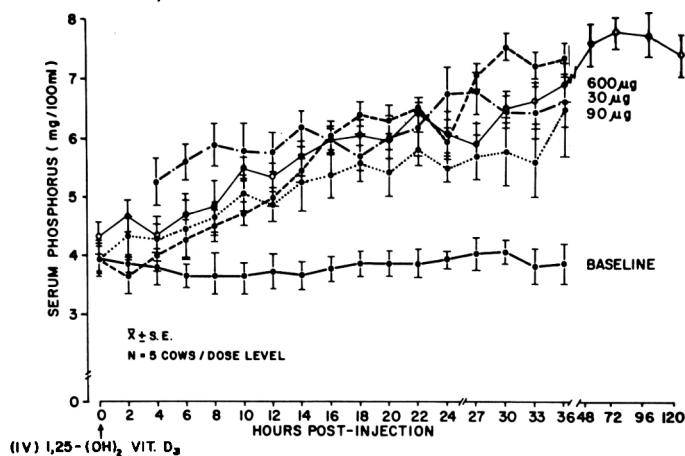EFFECT OF 1,25-DIHYDROXYCHOLECALCIFEROL ON SERUM PHOSPHORUS



Figure 2. Changes in serum phosphorus for 36 hours following administration of 30, 90, 270, and 600 μg 1,25-(OH)₂D₃ intravenously to cows compared to baseline values. Cows administered 600 μg of 1,25-(OH)₂D₃ were followed an additional four days to determine the persistence of the elevation in serum phosphorus.

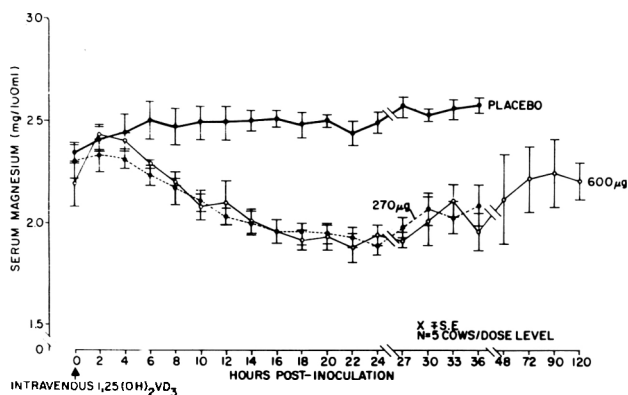EFFECT OF 1,25-DIHYDROXYCHOLECALCIFEROL ON SERUM MAGNESIUM



Figure 3. Depression of serum magnesium levels in cows following intravenous administration of 270 and 600 μg 1,25-(OH)₂D₃ compared to placebo-injected controls.

Serum magnesium levels were depressed (P<.05) by the intravenous administration of either 600 or 270 μg of 1,25-(OH)₂D₃ at six hours compared to placebo-injected controls (Figure 3). The decrease in magnesium persisted with the high dose of steroid until the experiment was terminated at 120 hours.

Urinary hydroxyproline was quantitated in cows receiving the high dose of 1,25-(OH)₂D₃ as an index of bone matrix catabolism. There was a significant elevation of hydroxyproline in cows given 600 μg 1,25-(OH)₂D₃ intravenously at 14, 22, 24, 30, 33, and 36 hours (Figure 4). These findings suggest that the active metabolite of vitamin D increased bone resorption in adult cows, in addition to its well-known effect on stimulating intestinal calcium transport (Figure 4). Urinary calcium, expressed as percent change from baseline, was consistently increased in cows receiving all four dose levels of 1,25-(OH)₂D₃ compared to baseline levels. However, changes in urine calcium did not correlate directly with the intravenous dose of steroid. Changes in urinary

phosphorus were less evident than changes in calcium; however, the two higher dose levels of steroid (270 and 600 μg) increased phosphorus excretion after 24 hours compared to baseline values.

The administration of 1,25-(OH)₂D₃ by the intramuscular route also resulted in a dose-related increase in serum calcium above placebo-injected controls but the response was slower than following intravenous injection. The earliest significant (P<.01) increase occurred at 18 hours in cows receiving 600 and 270 μg of steroid, and the blood calcium remained elevated during the five-day experimental period (Figure 5). A peak increase of 4.2 mg in calcium to 12.1 mg/dl occurred at 48 hours in cows administered 600 μg of steroid. Serum phosphorus was increased (P<.001) by 12 hours following intramuscular injection of 600 μg 1,25-(OH)₂D₃ and remained elevated during the 120-hour experimental period. A peak increase of 3.1 mg in phosphorus to 8.8 mg/dl occurred in cows receiving the high dose of steroid at 66 hours (Figure 6). Serum magnesium was decreased by 12 hours following 270 and 600 μg of 1,25-(OH)₂D₃ and remained low during the five-day experimental period (Figure 7).

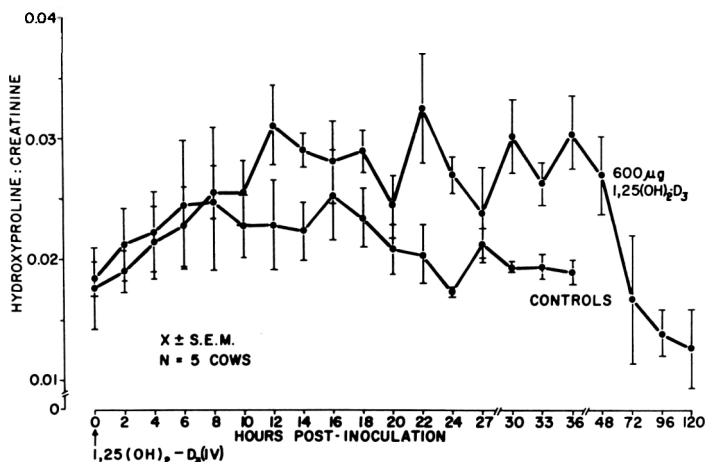EFFECT OF 1,25-DIHYDROXYCHOLECALCIFEROL ON URINARY HYDROXYPROLINE



Figure 4. Urinary hydroxproline (HOP) excretion, expressed as a ratio to creatinine, for 36 hours following administration of 600 μg 1,25-(OH)₂D₃ intravenously to cows compared to placebo-injected controls. Cows administered 600 μg 1,25-(OH)₂D₃ were followed an additional four days to determine the persistence of the effect on HOP excretion.

Urinary hydroxyproline was elevated (P<.001) by 12 hours following intramuscular injection of 1,25-(OH)₂D₃ and remained elevated for 48 hours (Figure 8). This increase occurred earlier than following intravenous administration of steroid, when the first significant (P<.05) elevation was detected at 14 hours.

1 alpha-hydroxycholecalciferol (1α-OH Vit. D₃) is a more readily synthesized analog of 1,25-dihydroxycholecalciferol that requires hydroxylation only in the liver. Following intramuscular injection of similar doses to the same cows, 1α-OH Vit. D₃ had much less effect on serum calcium than 1,25-(OH)₂D₃. The
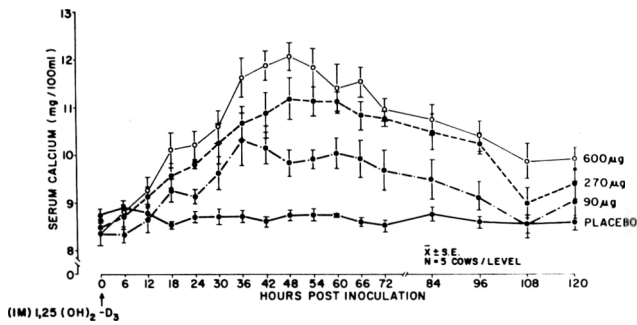
EFFECT OF 1,25-DIHYDROXYCHOLECALCIFEROL ON SERUM CALCIUM



Figure 5. Changes in serum calcium for 120 hours following administration of 90, 270, and 600 μg 1,25-(OH)2D3 intramuscularly in an oil vehicle to cows compared to cows receiving only placebo. Serum calcium was significantly elevated (P<.01) by 18 hours following intramuscular injection of 270 μg and 600 μg of 1,25-(OH)2D3 and remained elevated above placebo-injected controls during the 120 hour observation period. Serum calcium of cows administered 600 μg increased from 8.35 ± 0.24 mg/dl to a peak value of 12.06 ± 0.2 mg/dl at 48 hours post-injection.

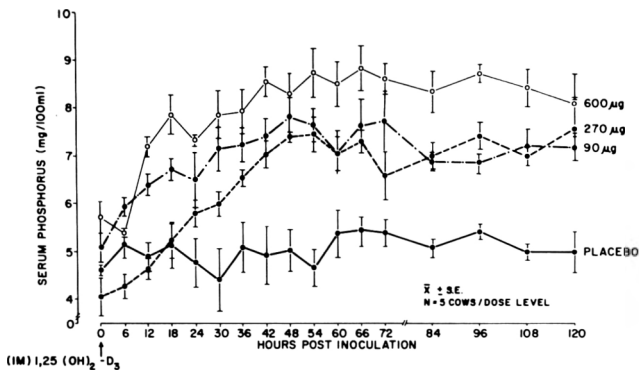EFFECT OF 1,25 DIHYDROXYCHOLECALCIFEROL ON SERUM PHOSPHORUS



Figure 6. Changes in serum phosphorus for 120 hours following administration of 90, 270, and 600 μg 1,25-(OH)2D3 intramuscularly in an oil vehicle to cows compared to cows receiving only placebo. Serum phosphorus was significantly elevated (P<.001) by 12 hours following intramuscular injection of 600 μg of 1,25-(OH)2D3 and remained elevated above placebo-injected controls during the 120-hour observation period.

earliest significant increase of serum calcium did not occur until 60 hours following injection of 600 μg of steroid (Figure 9). Serum phosphorus was elevated significantly above baseline values at 54 hours following intramuscular injection of either 270 μg (P<.001) or 600 μg (P<.05) of 1α-OH Vit. D3 (Figure 9). The latent period was longer and magnitude of effect on phosphorus was considerably less than following the intramuscular injection of a similar dose of 1,25-(OH)2D3 (Figures 2 and 6). 1α-hydroxycholecalciferol and 1,25-(OH)2D3 had a similar effect of decreasing serum magnesium following intramuscular injection. However, the first decrease appeared earlier (at 12 hours) and was of a greater magnitude following the administration of either 270 or 600 μg of 1,25-(OH)2D3 than 1α-OH Vit. D3. 1α-hydroxycholecalciferol resulted in an elevation (P< .05) in urinary hydroxyproline at 12, 18, 60, 72, and 96 hours following intramuscular injection. These findings suggest that 1α-OH Vit. D3 also was capable of

increasing bone resorption in addition to the known action of augmenting intestinal calcium transport. The reason why 1α-OH Vit. D3 was less effective in elevating serum calcium and phosphorus than 1,25-(OH)2D3 in the same adult cows is uncertain.

**The results of this dose-response study demonstrated that relatively small doses of 1,25-(OH)2D3 produced a rapid and persistent increase in serum calcium and phosphorus in dairy cows when administered either by the intravenous or intramuscular route. The short interval preceding a significant elevation in blood calcium and ability to control more precisely the magnitude of serum calcium increase, thereby minimizing toxicity, were considered to be definite advantages of 1,25-(OH)2D3 compared to the parent vitamin D3 compound in the development of a more effective prophylactic regimen for parturient hypocalcemia-susceptible cows. Similar doses of 1α-OH Vit. D3 were much less effective in elevating the serum calcium and phosphorus in the same adult cows.**

## Effect of 1,25-Dihydroxycholecalciferol in Pregnant Cows

The objective of this part of the investigation was to determine a prepartal dose schedule for 1,25-(OH)2D3 administration, based upon data from the dose-response study, that was effective in preventing the profound decline in serum calcium and phosphorus near parturition and development of parturient paresis. Fourteen pregnant Jersey cows, five years of age or older, were selected from a purebred herd with a high incidence of parturient paresis near the time of parturition. The cows had a previous history of developing parturient paresis at one or more preceding parturitions that often had required multiple injections of organic calcium solutions to correct the disturbance of calcium homeostasis. Data based upon several different dose schedules for 1,25-(OH)2D3 administration to pregnant cows were

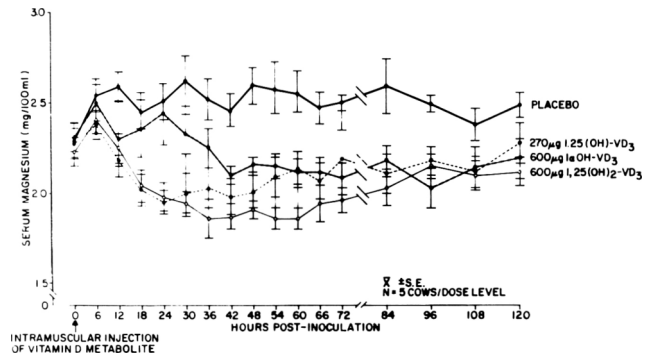EFFECT OF 1,25 DIHYDROXYCHOLECALCIFEROL AND 1,α HYDROXYCHOLECALCIFEROL ON SERUM MAGNESIUM



Figure 7. Comparative effects of 1,25-(OH)2D3 (270 and 600 μg) and 1α-OH Vit. D3 (600 μg) on serum magnesium in non-lactating cows. Although both steroids depressed magnesium levels, the changes appeared earlier (12 hours) and were of greater magnitude following 1,25-(OH)2D3.
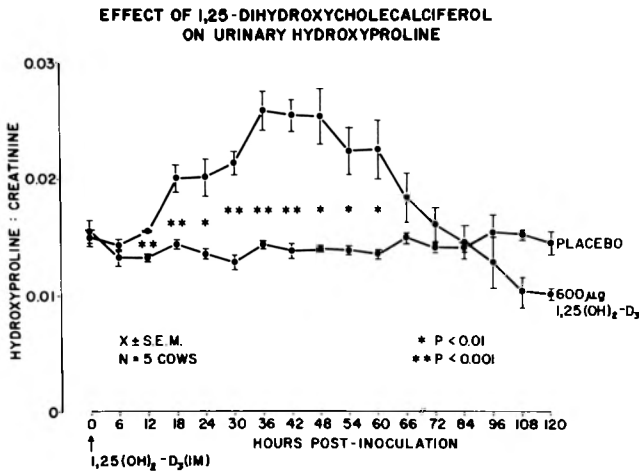
EFFECT OF 1,25-DIHYDROXYCHOLECALCIFEROL
ON URINARY HYDROXYPROLINE

Figure 8. Urinary hydroxyproline excretion in cows administered 600 μg of 1,25-(OH)₂D₃ intramuscularly compared to placebo-injected controls. There was a significant elevation (P<.001) of hydroxyproline excretion, expressed as a ratio to creatinine, in cows administered 600 μg of 1,25-(OH)₂D₃ at 12 hours post-injection which persisted for 48 hours.

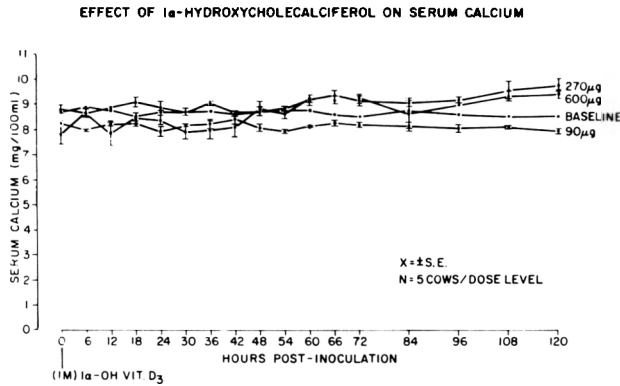EFFECT OF 1α-HYDROXYCHOLECALCIFEROL ON SERUM CALCIUM

Figure 9. Changes in serum calcium following intramuscular injection of 1α-OH Vit. D₃ (90, 270, 600 μg) in non-lactating cows. The earliest significant increase in calcium was at 60 hours following injection of the 600 μg dose.
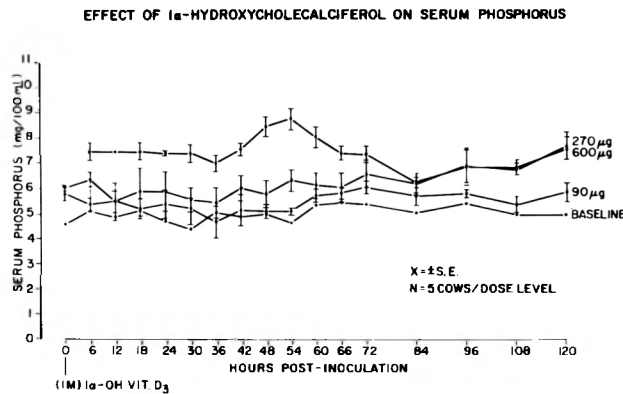
EFFECT OF 1α-HYDROXYCHOLECALCIFEROL ON SERUM PHOSPHORUS

Figure 10. Changes in serum phosphorus following intramuscular injection of 1α-OH Vit. D₃ (90, 270, 600 μg) in non-lactating cows. The earliest significant increase occurred at 54 hours following injection of either the 270 or 600 μg dose.

evaluated due to the inherent difficulties in precisely determining the hour of parturition. An initial dose of 600 μg of 1,25-(OH)₂D₃ was injected intramuscularly as close to the estimated date of parturition as possible or when the blood calcium concentration began to decline prior to parturition. Time of parturition was estimated by observation of udder filling and edema, and by swelling and relaxation of the vulva and pelvic ligaments. The cervix was palpated per vagina daily or every second day for evidence of relaxation and/or dilatation. Sequential changes in blood calcium, phosphorus, and magnesium were determined at six-hour intervals following the injection of 1,25-(OH)₂D₃. The diet consisted of ad libitum mixed hay and eight pounds of a commercial dairy concentrate mixture daily. Cows were not milked at the first regular milking period following parturition but were milked at regular 12-hour intervals thereafter.

The injection of 600 μg 1,25-(OH)₂D₃ intramuscularly in pregnant cows at 24 and 28 hours prepartum, when the blood calcium level was within the physiological range, was effective in preventing the development of a profound decrease in blood calcium and phosphorus near parturition (Figure 10). The blood calcium and phosphorus concentrations were relatively stable or increasing at parturition and in the immediate postpartal period during the initiation of lactation (Figure 10).

The date of parturition was misjudged in seven cows and a 600 μg dose of 1,25-(OH)₂D₃ was administered from 3.5 to 11.25 days prior to the actual time of parturition. Blood calcium and phosphorus levels were monitored at six-hour intervals and were stabilized by either the intramuscular or intravenous injection of smaller doses (270 μg) of 1,25-(OH)₂D₃ at approximately 48 to 96 hour intervals until the actual time of parturition. The blood calcium infrequently and for only short intervals exceeded 12 mg/dl and there was no clinical evidence of toxicity in pregnant cows administered from two to four doses of 270 μg of 1,25-(OH)₂D₃.

Figure 11 illustrates the changes in blood calcium, phosphorus, and magnesium following the administration of a 600 μg and a 270 μg dose of 1,25-(OH)₂D₃ separated approximately by a three-day interval. There was a progressive elevation of blood calcium for 60 hours following administration of the larger dose of steroid. The serum calcium was 10.9 mg/dl at parturition and remained at 9.3 mg/dl 42 hours postpartum. The serum phosphorus was 6.2 mg/dl at parturition and had increased to 7.2 mg/dl at 42 hours postpartum.

The data from a seven-year-old Jersey in Figure 12 is representative of a cow receiving three injections of 1,25-(OH)₂D₃ in which the serum calcium had decreased to 5.5 mg/dl at approximately eight days prior to parturition. Following the intramuscular injection of 600 μg 1,25-(OH)₂D₃ the serum calcium had increased to 10 mg/dl by 78 hours. The subsequent injection of two intravenous doses of 270 μg 1,25-(OH)₂D₃ at 3.75 and 1.5 days prepartum progressively

EFFECT OF I,25 DIHYDROXYCHOLECALCIFEROL ON SERUM CALCIUM, PHOSPHORUS,
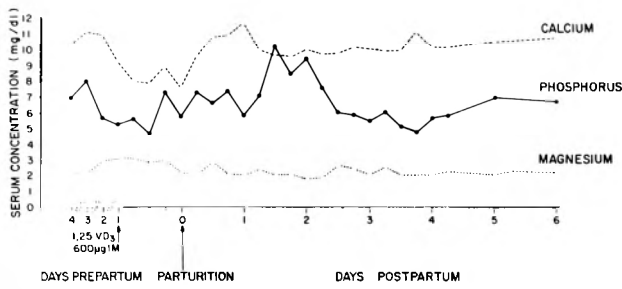AND MAGNESIUM IN A PREGNANT JERSEY COW



Figure 11. Effect of a single intramuscular injection of 600 μg 1,25-(OH)2D3 on stabilizing serum calcium, phosphorus, and magnesium through parturition and six days postpartum in a parturient paresis-susceptible pregnant Jersey cow.

EFFECT OF I,25-DIHYDROXYCHOLECALCIFEROL ON SERUM CALCIUM, PHOSPHORUS AND MAGNESIUM
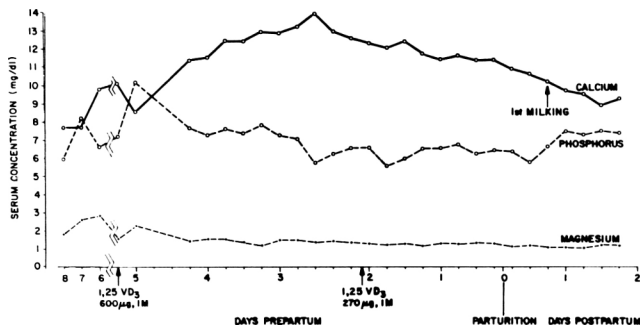IN A PREGNANT JERSEY COW



Figure 12. Effect of two doses (600 μg, 270 μg) 1,25-(OH)2D3 separated by a three-day interval on serum calcium, phosphorus, and magnesium in a parturient paresis-susceptible pregnant Jersey cow. The serum calcium was 10.9 mg/dl at parturition and remained at 9.3 mg/dl at 42 hours postpartum.

EFFECT OF I,25 DIHYDROXYCHOLECALCIFEROL ON SERUM CALCIUM,
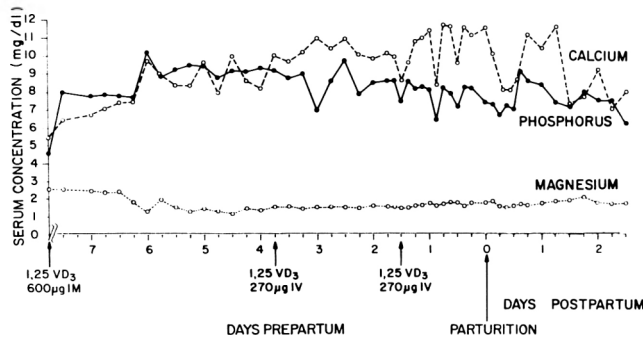PHOSPHORUS, AND MAGNESIUM IN A PREGNANT JERSEY COW



Figure 13. Effect of three doses (600 μg, 270 μg, 270 μg) 1,25-(OH)2D3 on serum calcium, phosphorus, and magnesium in a parturient paresis-susceptible pregnant Jersey cow. The initial dose (600 μg) was administered approximately eight days preceding parturition when the serum calcium had declined to 5.5 mg/dl. Electrolyte values were maintained in the physiologic range at parturition and 60 hours postpartum.

elevated the serum calcium to 11.4 mg/dl at parturition. Serum calcium, phosphorus, and magnesium levels remained in the physiologic range during the 60-hour observation period following parturition (Figure 12).

In three cows the initial dose of steroid was delayed until 8, 16 and 18 hours prior to the actual time of parturition, when the serum calcium had declined to between 5.0 - 5.5 mg/dl. A single 600 μg intramuscular dose of 1,25-(OH)2D3 was unable to return the serum calcium and phosphorus levels to the physiologic range when administered between 8 and 18 hours prepartum (Figure 13). Decreased gastrointestinal motility and anorexia associated with the lowered blood calcium appeared to interfere with the effectiveness of the 1,25-(OH)2D3. The serum calcium declined below 4 mg/dl and phosphorus below 2 mg/dl at parturition, and clinical signs of parturient paresis were evident at four hours postpartum. However, the administration of 16.8 gm calcium and 9.6 gm phosphorus restored electrolyte homeostasis in cows that during previous parturitions had required multiple injections of organic calcium solutions.

## Preliminary Study on Long-Term Effects of 1,25-Dihydroxycholecalciferol in a Pregnant Cow

In one Jersey cow the initial dose of 600 μg of 1,25-(OH)2D3 was given at the time of estimated date of parturition reported by the owner. Although parturition did not appear to be imminent clinically, the owner stated that in previous years the cow did not fill the mammary gland or develop other clinical signs of impending parturition until one day prior to delivery. The cow actually delivered 22 days after the initial injection of 1,25-(OH)2D3. The owner had evidently missed a breeding at the following estrus cycle. Because of the misjudged date, this cow afforded an excellent opportunity to determine the possible toxic effects of long-term administration of 1,25-(OH)2D3 to a parturient paresis-susceptible pregnant cow.

A total of seven doses of 1,25-(OH)2D3 (one dose at 600 μg and six doses at 270 μg) were given at 48 to 96 hour intervals. The total dose of 1,25-(OH)2D3 administered to this cow was 2,200 μg. The serum calcium exceeded 12 mg/dl at only six intervals during the 22 day period reaching a peak of 12.6 mg/dl at 48 hours prior to parturition (Figure 14). The serum phosphorus was moderately elevated during the first 13 days but subsequently declined over the last nine days prepartum. There was a slowly progressive decline in serum magnesium from 2.0 mg/dl to 0.5 mg/dl over the 22-day period.

During the 22-day period the cow ate little and developed persistent ketosis. Serum sorbitol dehydrogenase levels ranged from 25.9 to 43.2 IU/l (normal values range up to 15 IU/l). These findings were interpreted to suggest a clinical diagnosis of pregnancy toxemia associated with fatty degenera-
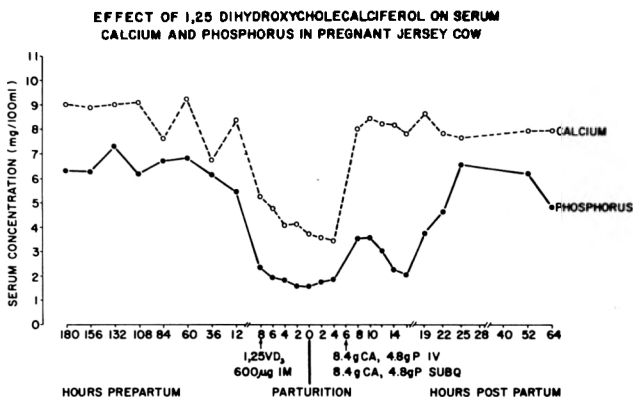
EFFECT OF 1,25 DIHYDROXYCHOLECALCIFEROL ON SERUM
CALCIUM AND PHOSPHORUS IN PREGNANT JERSEY COW

Figure 14. Effect of a single dose (600 μg) of 1,25-(OH)₂D₃ administered intramuscularly eight hours prior to the actual time of parturition in a parturient paresis-susceptible Jersey cow. The serum calcium was approximately 5 mg/dl at the time of 1,25-(OH)₂D₃ injection and it continued to decline at parturition, requiring administration of an organic calcium solution.
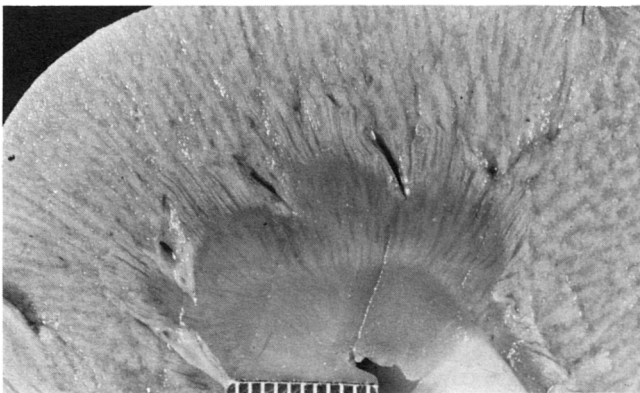


EFFECT OF 1,25 DIHYDROXYCHOLECALCIFEROL ON SERUM CALCIUM, PHOSPHORUS AND MAGNESIUM IN A
PREGNANT JERSEY COW

Figure 15. Effect of seven doses of 1,25-(OH)₂D₃ (1-600 μg, 6-270 μg) on serum calcium, phosphorus, and magnesium administered over 22 days and separated by intervals of 48 to 96 hours to a parturient paresis-susceptible pregnant Jersey cow. The serum calcium exceeded 12 mg/dl at only six intervals, reaching a peak of 12.6 mg/dl at 48 hours prepartum.



Figure 16. Kidney from pregnant cow receiving seven injections of 1,25-(OH)₂D₃ (total dose - 2,200 μg) illustrating lack of renal mineralization.
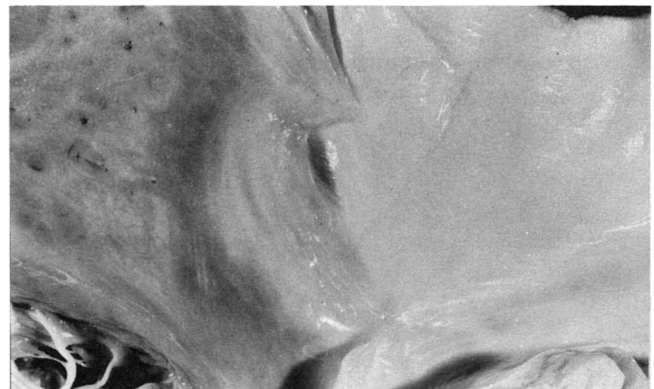


Figure 17. Pulmonary artery and right ventricle from a pregnant cow receiving seven doses of 1,25-(OH)₂D₃ (total dose - 2,200 μg) with no macroscopic evidence of cardio-vascular mineralization. Histopathologic evaluation of multiple areas of major vessels and heart failed to reveal evidence of mineralization following long-term administration of 1,25-(OH)₂D₃.

tion of the liver. The cow also developed a left displacement of the abomasum immediately prior to delivery.

A complete necropsy was performed one day after parturition. Detailed macroscopic and histopathologic examination failed to demonstrate evidence of mineralization in the kidney (Figure 15), heart (Figure 16), pulmonary artery (Figure 16), abdominal aorta (Figure 17) or other soft tissues as has been reported following long-term administration of the parent vitamin D compound (3). The liver had extensive fatty infiltration and there was a fibrinous thrombus attached to the wall of the anterior vena cava, apparently the result of the intravenous catheter. These lesions were interpreted to be unrelated to the long-term administration of 1,25-(OH)₂D₃.
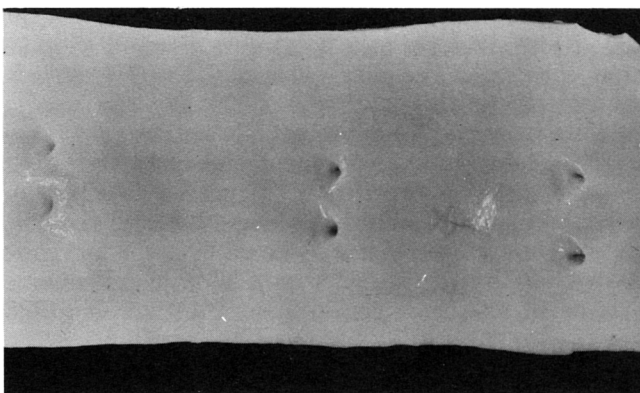


Figure 18. Normal abdominal aorta from pregnant cow receiving seven doses of 1,25-(OH)₂D₃ (total dose - 2,200 μg). There was no evidence of soft tissue mineralization following the long-term (22 day) administration of the active vitamin D metabolite.

## Summary
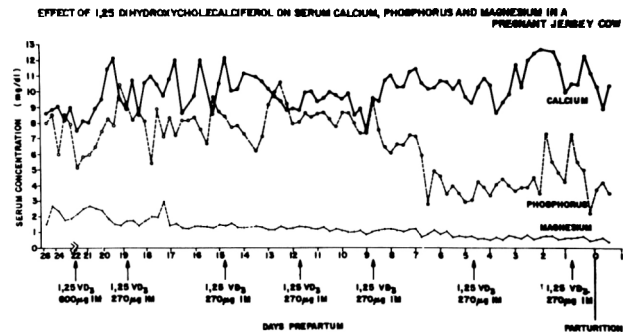
1,25-dihydroxycholecalciferol administered either intramuscularly or intravenously to adult cows resulted in a rapid and persistent, dose-dependent elevation in serum calcium and phosphorus, and a decline in serum magnesium. The significant eleva-

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tion in urinary hydroxyproline suggested the steroid increased bone resorption in adult cows. 1α-hydroxycholecalciferol administered intramuscularly to the same group of cows at identical dose levels resulted only in a mild and transient increase in serum calcium and phosphorus, but a persistent decrease in serum magnesium. The administration of 600 μg 1,25-(OH)$_2$D$_3$ to pregnant cows 24 or more hours prepartum prevented the profound decline in serum calcium and phosphorus near parturition. Cows in which the actual hour of parturition was not predicted received an initial injection of 600 μg of 1,25-(OH)$_2$D$_3$ followed by repeated doses of 270 μg of steroid to sustain the stabilization of serum calcium and phosphorus. The more rapid onset of action and ability to more precisely regulate the magnitude of elevation in blood calcium, thereby minimizing potential toxic effects, are distinct advantages of 1,25-(OH)$_2$D$_3$ over the parent vitamin D compound in the development of an effective prophylactic regimen for parturient paresis-susceptible dairy cows.

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