Relationships between prepartal dietary phosphorus and calcium in vitamin D metabolism and incidence of parturient paresis in Jersey cows

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INTRODUCTION

Initiation of lactation imposes a sudden, severe increase in the calcium and phosphorus needs of the dairy cow. When compensatory mechanisms do not respond sufficiently to prevent plasma calcium and phosphate concentrations from decreasing below critical levels, parturient paresis (milk fever) occurs.

Although many factors influence the development of parturient paresis, the following sequence of events perhaps best describes the main etiologic cause of parturient paresis. Because of decreased demands for calcium and phosphorus during the dry period, the cow relies primarily on dietary sources of these two minerals to meet her needs. Consequently, the homeostatic mechanisms of the bone and gut become relatively inactive. As lactation is initiated, the previous rate of dietary influx of calcium is no longer adequate to meet the calcium demands and, if the bone and gut homeostatic mechanisms cannot respond quickly enough to meet the increased demands, parturient paresis develops.

1,25-Dihydroxyvitamin D (1,25-$(OH)_2D$), a biologically active form of vitamin D, increases the efficiency of absorption of calcium and phosphorus from the gut (DeLuca, 1979). 1,25-Dihydroxyvitamin D, together with parathyroid hormone (PTH), stimulates calcium and phosphorus mobilization from bone (Raisz, 1963, Raisz et al., 1972a). A prepartum, low calcium diet (below daily calcium requirements) was shown to increase plasma PTH concentrations during the prepartal period and to prevent parturient paresis (Goings et al., 1974). Green et al. (1981) recently has presented evidence that a prepartum, low calcium diet stimulates the production of 1,25-$(OH)_2D$.

Thus, these increases in PTH and 1,25-$(OH)_2D$ would activate the compensatory mechanisms of the bone and gut prepartally and provide for effective calcium homeostasis following parturition. Tanaka et al. (1973) have shown that a low phosphorus diet (below daily phosphorus requirements) will increase plasma 1,25-$(OH)_2D_3$ concentrations and calcium absorption in the gut of the rat. Boda (1956) prevented parturient paresis by feeding a high phosphorus, low calcium, diet prepartum. He proposed that this dietary combination effectively stimulated PTH secretion by inducing a state of nutritional secondary hyperparathyroidism in the cows.

The present study was undertaken to clarify the role that different amounts of prepartal dietary phosphorus might play in vitamin D metabolism and the prevention of parturient paresis. Interactions between dietary phosphorus and calcium were investigated. It is hoped that the results obtained from this study will lead to a clearer understanding of vitamin D metabolism in dairy cattle and of the mechanism by which various dietary regimes prevent parturient paresis.

REVIEW OF LITERATURE

Changes in several blood constituents occur to varying degrees in all cows at parturition (Littledike et al., 1969). Many of these changes are most pronounced in cows with parturient paresis.

Hypocalcemia is recognized as the primary cause of the observable symptoms of parturient paresis (Little and Wright, 1925; Mayer et al., 1969a; Littledike, 1974). Although most periparturient cows exhibit some degree of hypocalcemia, parturient paresis and its associated symptoms of hyperexcitability, weakness, and incoordination usually do not occur until plasma calcium falls to less than 5 mg/100 ml (normal plasma concentrations range from 8.5 to 11.4 mg/100 ml (Little, 1932)). In the latter stages of the disease, the cow becomes recumbent, her skeletal muscles become flaccid, and she may become comatose (Hibbs, 1950).

Intravenous administration of a solution of calcium salts is the usual treatment when the cow becomes recumbent. Treatment usually results in complete recovery in as little as 15 minutes (Mayer et al., 1969a), but 10-30% of the cows relapse (Mayer et al., 1969a; Jönsson, 1978). Even when successfully treated, cows that develop parturient paresis lose a significant portion of their productive life (Payne, 1964).

Parathyroid hormone, secreted in response to a decrease in plasma calcium concentration, acts to increase plasma calcium by stimulating bone resorption (Barnicot, 1948; Raisz, 1963), increasing 1,25-$(OH)_2D$ production (Fraser and Kodicek, 1973), and improving calcium reabsorption, either directly or indirectly, in the kidney (DeLuca, 1979). Consequently, one of the first theories on the etiology of parturient paresis emphasized the

possibility of insufficient PTH response to the developing hypocalcemia (Dryerre and Greig, 1928). This controversial theory had much experimental evidence against it (Stott and Smith, 1957; Mayer et al., 1967; Capen and Young, 1967); the theory was not directly disproved until recently. Mayer et al. (1969b) and Horst et al. (1978) showed both nonparetic and paretic cows to have increased PTH levels in response to hypocalcemia at parturition.

Because 1,25-$(OH)_2D$ also is secreted in response to hypocalcemia and acts to increase calcium absorption from the gut and resorption from the bone, perhaps insufficient 1,25-$(OH)_2D$ response is a cause of parturient paresis. Horst et al. (1978) demonstrated increased levels of 1,25-$(OH)_2D$ in response to hypocalcemia in paretic cows. The discovery that paretic cows actually had higher 1,25-$(OH)_2D$ levels than nonparetic cows (200 vs. 100 pg/ml) led Horst and coworkers to theorize that target-organ resistance to 1,25-$(OH)_2D$ and PTH could be the cause of the disease. These findings support and extend the hypothesis presented by Kronfeld (1968). Target organ resistance could be promoted by intermittent exposure to calcitonin. Calcitonin, secreted in response to high concentrations of plasma calcium, inhibits bone resorption (Wallach et al., 1967). The typically high calcium prepartal diet of the cow may tend to increase plasma calcium concentrations and stimulate calcitonin secretion. Capen and Young (1967) showed parturient paretic cows to have less extractable calcitonin in their thyroid glands than did control cows. These findings suggest that paretic cows had secreted more calcitonin prepartum, which could result in bone being nonresponsive to PTH and 1,25-$(OH)_2D$ at parturition. Reinhardt and Conrad (1980a), based on their finding of 1,25-$(OH)_2D$ receptors in the

mammary gland of the cow (Reinhardt and Conrad, 1980b), suggest the high levels of 1,25-$(OH)_2D$ found in paretic cows could contribute to the development of hypocalcemia by causing an additional efflux of calcium from plasma to the mammary gland.

Milk contains one gram or more per liter of phosphorus (Mayer et al., 1969a), and, thus, initiation of lactation is likely to stress the phosphorus homeostasis of the cow. Hypophosphatemia occurs at parturition (Fish, 1929). Development of hypophosphatemia could be exacerbated through the phosphatemic action of the high levels of PTH found at parturition (Horst et al., 1978). Parathyroid hormone lowers plasma phosphate by decreasing reabsorption of phosphate in the kidney (Forte et al., 1976). When calcium salts, containing no phosphorus, are given for treatment of parturient paresis, both plasma calcium and phosphate levels increase (Fish, 1929). This could be explained by the increase in plasma calcium inhibiting PTH secretion and, thus, decreasing phosphate excretion in the urine.

Plasma phosphate concentrations are much more labile in response to changes in homeostasis than is plasma calcium. For instance, Fish (1929) found plasma phosphate concentrations of cows to average 4.65 mg/100 ml during lactation, compared to an average concentration of 6.25 mg/100 ml during the dry period. Plasma calcium concentrations, however, showed only a transient decrease in response to lactation. Plasma phosphate also increases more rapidly than plasma calcium in response to udder insufflation. Plasma calcium concentrations in chicks only varied from 9.2-10.4 mg/100 ml when a low or high calcium diet was fed (Haussler et al., 1977). However, when low or high dietary phosphorus diets were fed, plasma phosphate concentrations varied from 5.2-13.9 mg/100 ml. Thus, in general, plasma

phosphate concentrations are less strictly controlled and fluctuate more readily than does plasma calcium level.

A number of other constituents of plasma change at parturition and many of these changes seem associated with the developing hypocalcemia and hypophosphatemia (Littledike et al., 1969). Some of these changes were increases in concentrations of magnesium, hydrocortisone, glucose, chloride, hydroxyproline, and L-lactate and decreases in concentrations of plasma insulin (Littledike et al., 1969).

Changes in plasma progesterone and estrogen concentrations around the time of parturition are independent of the developing hypocalcemia. Plasma progesterone concentrations decrease sharply just before parturition, while plasma estrogens increase (Smith et al., 1973). The observation that estrogens stimulate bone accretion (Ranney, 1959a) led to the theory that paretic cows might have greater concentrations of estrogens or an imbalance in the progesterone/estrogens ratio compared to normal parturient cows (Bargeloh et al., 1975). Lintner (1977), however, showed no difference between paretic and nonparetic cows with respect to plasma progesterone and estrogen concentrations at parturition.

Factors Influencing Incidence of Parturient Paresis

Although parturient paresis is generally reported as occurring in 5-10% of cows (Jorgensen, 1974, Jönsson, 1978), the incidence will vary depending on a number of factors, including breed, production, age, individual predisposition, previous incidence, and diet. It is the combination of these factors that determines the extent of hypocalcemia at parturition.

Breed

It appears that parturient paresis was unknown before the 18th century, but was not mentioned in the literature until 1793 (Hutyra et al., 1938). Incidence of the disease has been increasing since that time, parelleling increases in milk production. Jönsson (1960) reported an increase in parturient paresis incidence from 0.6% in 1937 to 2.36% in 1955. In a more recent study, Jönsson (1978) stated that the incidence of parturient paresis has doubled since 1950 and presently affects 8% of dairy cows in Sweden. Payne (1968) reported a 3.54% and a 2.96% incidence in 1957-58 and 1958-59, respectively, in Great Britain. Metzger and Morrison (1936) reported a 3.9% incidence in Holstein cows and 12.4% in Jersey cows in the United States. Henderson (1938) showed a 29.2% incidence in Jersey cows, 8.6% in Guernsey cows, 6.0% in Ayrshire cows, 15.3% in Brown Swiss cows, and 13.3% in Shorthorn cows. There are recent reports that incidence of parturient paresis in Holstein cows ranges from 8-10% (Jorgensen, 1974) to 19% (Gardner and Park, 1973) and, in some herds, as high as 70% (Jorgensen, 1972). It is likely that as the average level of milk production in dairy cows increases in the future, incidence of the disease may increase further.

Milk production

Factors other than level of milk production may be a more important determinant in development of parturient paresis. It is difficult to separate the effects of milk production from age because milk production generally increases with age. Jönsson (1960) showed that cows which developed the disease had greater annual milk production regardless of the number of previous lactations. However, Hibbs (1950) stated that many cows that

developed parturient paresis were not outstanding producers. In addition, Hibbs et al. (1951) found the amount of calcium secreted in the colostrum of the first 2 milkings by cows with parturient paresis was not greater than that of normal parturient cows. Payne (1968) showed a 5.7% incidence in cows beginning their 4th lactation and secreting 21.8 grams of calcium in the first 24 hours' milk compared to 8.2% incidence in cows beginning their 5th lactation and secreting only 16.3 g of calcium in the first 24 hours' milk. It seems possible that, although the initiation of lactation is obviously the precipitating factor leading to hypocalcemia, the precise level of milk production is not of major importance in the predisposition of cows to parturient paresis.

Age

More important than the level of milk production is the cow's relative ability to adjust to the imbalance in homeostasis created by the initiation of lactation. This adaptability is influenced greatly by the age of the cow. Parturient paresis is more prevalent in older cows (Hibbs, 1950). Marr et al. (1955) found the incidence of parturient paresis to increase until at least the 4th calving. Payne (1968) found only a 0.2% incidence in 1st lactation heifers and a 0.7% incidence in 2nd lactation cows. Incidence increased through the 6th lactation to 9.6%. Jönsson (1960) also found low incidence in 1st and 2nd lactation cows. Jorgensen (1972) stated that herds in Wisconsin frequently have 50% or greater incidence in cows with 3 or more lactations.

The cow's ability to supply calcium for milk decreases with age for a number of reasons. Older cattle are less efficient in absorbing calcium from

the gut (Hansard et al., 1954a), excrete greater endogenous calcium (Hansard et al., 1954a), and have less exchangeable bone (Hansard et al., 1954b) than do younger cattle. Paretic cows tend to be in negative calcium balance for about 2 weeks prepartum, but older cows which do not develop paresis are borderline and 1st calf heifers are in positive balance (Ward et al., 1952). Plasma hydroxyproline concentration is a useful index of bone turnover (Black and Capen, 1971). Older cows had lower concentrations of hydroxyproline than did younger cows, which would indicate that there is a decreased calcium mobilization from bone with age (Evans et al., 1976).

All of these findings indicate that older cows have more difficulty adjusting to the calcium demands at parturition than do younger cows, and, thus, are more susceptible to parturient paresis.

Once a cow develops parturient paresis, she is more likely to get the disease at subsequent parturitions (Hibbs, 1950; Jorgensen, 1972).

Prepartal diet

Prepartal diets influence the incidence of parturient paresis (Stott, 1968). Gardner and Park (1973) and Beitz et al. (1974) found that prepartal diets high in calcium increase incidence of the disease. Others, including Boda and Cole (1954) and Goings et al. (1974) showed that prepartal diets low in calcium effectively prevent the disease. High dietary intake of calcium by prepartal cows was associated wtih lower PTH content in the parathyroid glands, decreased secretory activity of the gland, and decreased response to a plasma calcium challenge (Rowland et al., 1972; Black et al., 1973). Belyea et al. (1976) found low calcium diets improved the ability of cows to respond to an EDTA challenge. Cows fed a low calcium

diet also had greater urinary hydroxyproline concentrations, greater percentage of bone surfaces undergoing resorption, and greater numbers of parathyroid gland chief cells in the actively synthesizing phase than did cows fed an adequate calcium diet (Yarrington et al., 1977). Green et al. (1981) showed that cows fed a low calcium diet had greater prepartal plasma 1,25-$(OH)_2D$ concentrations than did cows fed a high calcium diet. The results of these dietary studies indicate that the amount of prepartal dietary calcium determines whether a cow's calcium homeostatic mechanisms are in an "active" state (low calcium diet) or a passive state (high calcium diet) and this will influence whether or not the cow will be able to adjust to the calcium demands at parturition.

The influence of dietary phosphorus on incidence of parturient paresis is less clear. Boda and Cole (1954) suggested prepartal diets high in phosphorus caused a hyperactive parathyroid gland, and, when in combination with low dietary calcium, provided the best preventative measure against parturient paresis. Their diet, although effective in reducing the incidence of the disease, actually contained presently recommended amounts of phosphorus (National Research Council, 1978). On the other hand, Tanaka et al. (1973) showed high phosphorus to interfere in the conversion of 25-hydroxyvitamin D (25-OHD) to 1,25-$(OH)_2D$. If this were the case in cows, high dietary phosphorus would be counterproductive in preventing the disease.

Other studies (Kendall et al., 1970; Gardner and Park, 1973) have emphasized the importance of a 2.3:1 calcium to phosphorus ratio in the diet in preventing the disease. Results by Beitz et al. (1974) indicate that

the relative amounts of calcium and phosphorus are more important than the ratio in preventing parturient paresis.

Estrogens

Estrogens increase dramatically near parturition in the plasma (Smith et al., 1973) and urine (Mellin et al., 1965) of cows. Estrogens stimulate bone uptake of plasma calcium in the rat (Ranney, 1959a) and also might act in opposition to parathyroid hormone by inhibiting bone resorption (Ranney, 1959b; Riggs et al., 1969). Thus, high levels of estrogens in plasma at the time of parturition could act to antagonize the cow's attempt to increase calcium mobilization from bone in response to lactation.

Moodie and Robertson (1962) found that feed intake and gut motility decreased at calving time. Experimentally induced gut stasis reduced feed intake and caused hypocalcemia. Administration of estradiol-17β from 7 days before projected parturition date until parturition decreased feed intake of the treated cows compared to control cows (Bargeloh et al., 1975). Muir et al. (1970) was able to counteract the appetite-depressant effect of estrogen by injecting progesterone. As indicated by urinary hydroxyproline, there was no evidence of inhibited bone resorption in estrogen-treated cows. This was confirmed by work of Payne (1970) and Bargeloh et al. (1975).

In conclusion, high levels of estrogen in plasma around the time of parturition probably contribute to the developing hypocalcemia by contributing to inappetance and, thus, decreasing the availability of dietary calcium. It is questionable whether high levels of estrogen significantly inhibit bone resorption.

Individual predisposition

Until recently, the influence of the genetic component contributing to parturient paresis has received little attention. Dyrendahl et al. (1972) estimated the heritability of the disease to be 0.13. There is some degree of genetic control over calcium concentration in milk (Comberg and Meyer, 1963) and the degree of hypocalcemia around parturition (Graf and Osterkorn, 1976).

Jönsson et al. (1980) found a significant effect of the individual cow on incidence of parturient paresis. The individual also had a significant effect on prepartal and peripartal plasma concentrations of calcium, inorganic phosphorus, magnesium, hydroxyproline, and PTH. There was a significant negative correlation between calcium and PTH levels only for cows that did not develop parturient paresis. In this study, cows that developed parturient paresis actually had slightly greater concentrations of PTH prepartum than did cows that did not develop the disease. These results can be interpreted as lending support to the idea of a target organ resistance to calcium-mobilizing hormones such as PTH in cows predisposed to parturient paresis.

Variability of the individual's response to changes in calcium and phosphorus homeostasis also would aid in explaining breed differences in susceptibility and herd differences within the same breed. For example, some Holstein herds have incidences as high as 50%, even though the breed as a whole averages around 10% (Jorgensen, 1972).

Prevention of Parturient Paresis

PTH, vitamin D and its metabolites

Based on the parathyroid insufficiency theory, injections of PTH or PTH extract were among the first methods tried to prevent parturient paresis. Hibbs et al. (1947) and Jackson et al. (1962) found that injections of PTH extract around the time of parturition did not prevent parturient paresis. Cows receiving the PTH extract had no measurable response. Administration of PTH extract within two hours postpartum did not alter the concentrations of calcium or magnesium in the blood. Hibbs et al. (1947) suggested that PTH secretion was sufficient in paretic cows, but was rendered temporarily inactive by some metabolic condition in the tissues at parturition.

Vitamin D and its metabolites are intimately associated with the control of calcium and phosphorus homeostasis (DeLuca, 1979) and, as such, are obvious candidates for parturient paresis prevention.

Vitamin D is enzymatically converted to 25-OH D predominantly in the liver (Horsting and DeLuca, 1969). 25-Hydroxyvitamin D, the main circulating form of the vitamin, is a substrate for a 25-hydroxy-10-hydroxylase (1-hydroxylase) (DeLuca, 1979). Activity of the 1-hydroxylase is influenced by a number of factors that were summarized and reviewed recently by Fraser (1980). The main controlling factors include PTH, calcium, and possibly phosphorus.

Efforts to prevent parturient paresis have included the use of vitamin D, both orally and injected as vitamin D_2, 25-OH D injections, $1,25\text{-}(OH)_2D$

injections, and 1α-hydroxyvitamin D_3 (1,α-OH D_3) injections (a synthetic analog which is converted in the liver to 1,25-$(OH)_2D$).

Attempts to prevent parturient paresis with vitamin D date back to initial studies by Hibbs et al. (1946) in which the feeding of 1 million units per day of vitamin D_2 had no effect on incidence of the disease. Hibbs and Conrad (1960) decreased the incidence by 70-80% by feeding 20-30 million units of vitamin D_2 daily for 3 to 7 days before calving. Unfortunately, cows calving before having been fed the vitamin D_2 for at least 3 days were not protected. If fed for longer than 7 days, the vitamin D_2 may be toxic (Capen et al., 1968). Further difficulties arise if the treatment is discontinued for more than 1 day before parturition. Preventative effects disappear and incidence of parturient paresis actually may increase (Hibbs and Conrad, 1960). Continuous feeding of low levels of vitamin D_2 (70,400 units/kg grain) reduced incidence in cows with a previous history of the disease, but seemed to increase incidence in cows that had never had parturient paresis. Increased incidence after discontinuation of vitamin D_2 feeding and in cows with no previous history of the disease might be explained by work of Capen and Young (1969). They found that thyroid parafollicular cells of cows underwent hypertrophy within 5 days after vitamin D_2-induced hypercalcemia. These cell types seemed to increase secretion of calcitonin in response to the hypercalcemia. Calcitonin inhibits bone resorption (Friedman and Raisz, 1965). Thus, the bone of vitamin D_2-fed cows might be expected to be unresponsive at parturition if the vitamin D_2 feeding was discontinued. Additionally, the normally responsive bone of nonparturient paresis prone cows might become less responsive after exposure to calcitonin.

Large doses of vitamin D may increase the amount of circulating 25-OH D (Haschek et al., 1978). The hypercalcemia caused by high levels of vitamin D may decrease 1,25-$(OH)_2$D production because the synthesis of 1,25-$(OH)_2$D is regulated by the need for calcium and phosphorus (Fraser, 1980). Depressed levels of 1,25-$(OH)_2$D would result in decreased bone resorption and a low rate of calcium absorption from the gut. The supra-physiological levels of 25-OH D, which are dependent on vitamin D supply, act to increase bone resorption in vitamin D_2-fed cows. A cow would be in an "inactive" state of preparedness at the time of parturition if the vitamin D_2 feeding was withdrawn, and, thus, could increase incidence of the disease.

Intramuscular injections of 10 million units of vitamin D_2 given within 10 days before parturition provided some protection (Payne and Manston, 1967). This method still suffers from the same two major problems of oral doses of vitamin D_2: accurate prediction of calving date and danger of toxicity.

The use of 25-OH D as a preventative may have an advantage over that of vitamin D. Smaller and, thus, more accurate, amounts of 25-OH D can be given (4-8 ml), thus minimizing the danger of soft tissue calcification.

Intramuscular injections of 4 and 8 mg of 25-OH D produced a hypercalcemia of less magnitude and shorter duration than hypercalcemia induced by vitamin D injections (Jorgensen et al., 1978). This mild alteration would tend to lessen the secretion of calcitonin and avoid possible depression of

calcium mobilizing ability. There is a lag time of 72 hours before protection is afforded and a reduction in effectiveness if the cow calves more than 10 days post-injection (Jorgensen, 1974). Thus, 25-OH D, as a parturient paresis preventative, decreases somewhat the danger of toxicity, but still depends on precise timing for its effectiveness. Because of 25-OH D's persistance in plasma and extended biologic response, it would still present problems with toxicity. 25-Hydroxyvitamin D may depend on conversion to 1,25-$(OH)_2D$ for some of its effectiveness. Consequently, high amounts of dietary phosphorus will decrease the preventative effect of 25-OH D injections (Jorgensen, 1974).

Injections of 1,25-$(OH)_2D$ and 1,α-OH D_3 also have been studied for use as preventatives of parturient paresis. Hoffsis et al. (1978) found both intramuscular and intravenous injections of 1,25-$(OH)_2D$ caused dose-dependent hypercalcemia and hyperphosphatemia at 12-24 hours post-injection. Increases in urinary hydroxyproline after administration suggested the 1,25-$(OH)_2D$ had increased bone resorption. Gast et al. (1979) successfully prevented parturient paresis with intramuscular injections of 0.4 mg of 1,25-$(OH)_2D$ starting 5 days before the predicted calving date and repeated at 5-day intervals.

Intramuscular (IM) injections of 1,α-OH D_3 caused a gradual rise in plasma calcium and phosphorus concentrations that was characterized by an unpredictable lag time before response. As a result, in this study, IM injections of 1,α-OH D_3 were not as effective as intravenous (IV)

injections of 1α-OH D_3 in preventing parturient paresis (Gast et al., 1979).

Sansom et al. (1976) decreased the incidence of parturient paresis in Fresian cows with IM injections of 250 μg of 1,α-OH D_3 given within 2 hours after calving. Jönsson (1978), however, found doses of 0.5 μg and 1.0 μg/kg of body weight had no effect on plasma calcium and phosphorus concentrations. One milligram of 1,α-OH D_3 increased plasma calcium and phosphorus concentrations of cows, but also caused side effects, including severe soft tissue calcification (Jönsson, 1978). In contrast, no hyper-vitaminosis or soft tissue calcification with 1 mg injections of 1,α-OH D_3 was found by Gast et al. (1977).

Seemingly, the use of 1,25-$(OH)_2$D injections suffers from the disadvantage of high economic cost relative to other D metabolites. Injections of 1,α-OH D_3 not only depend on precise prediction of calving date, but similar to vitamin D injections, may cause serious side effects.

Variability in actual calving date limits the success of vitamin D metabolite injections. Recent workers have used corticosteroids in conjunction with 1,α-OH D_3 injections in an attempt to induce parturition during the time when 1,α-OH D_3 will be maximally effective in preventing parturient paresis. McMurray et al. (1980) and Bar et al. (1980) successfully prevented parturient paresis using this approach. Two hundred and fifty and 350 μg injections of 1,α-OH D_3, followed 2 days later by injections of 5 mg of flumethasone (which induced parturition within 24-48

hours), effectively prevented parturient paresis (Bar et al., 1980). Control cows had a 37% incidence.

Dietary methods

The influence of diet on incidence of the disease was noted in the early 1900s. Incidence of parturient paresis decreased in Europe during World War I when feed supplies were limited (Greig, 1930). Since that time, numerous investigators have attempted to prevent parturient paresis by changing some aspects of diet. Manipulation of the calcium to phosphorus ratio, absolute amounts of calcium, phosphorus, and magnesium, amount of grain, and alkalinity of the diet all have been tried and have shown varying degrees of success.

Absolute amounts of dietary calcium and phosphorus seem more important than the ratio of these 2 minerals in preventing parturient paresis (Beitz et al., 1974). Ender et al. (1956) discussed findings which, in summary, show prepartal high calcium diets to increase incidence of the disease. Conversely, low calcium diets effectively prevent parturient paresis (Boda and Cole, 1954; Goings et al., 1974; Wiggers et al., 1975). The amount of dietary calcium must be less than the cow's daily requirement to stimulate PTH secretion (Goings et al., 1974), bone mobilization (Yarrington et al., 1977), and 1,25-$(OH)_2D$ production (Green et al., 1981). Jönsson et al. (1980) found little influence on PTH levels when dietary intakes of calcium were between 37 and 150 g/day.

Efficiency of utilization of dietary calcium and phosphorus (percentage of dietary calcium and phoshporus absorbed) is inversely related to intake (Huffman et al., 1930; Ramberg and Kronfeld, 1971). This adaptation

to the amount of calcium in the diet is mediated by 1,25-$(OH)_2D$ (Omdahl and DeLuca, 1977).

In Europe, difficulties in feeding a diet containing less than 30 g of calcium per day have prompted development of alternative dietary methods. These methods, like the low calcium diets, have the common goal of stimulating the calcium homeostatic mechanisms that would respond to hypocalcemia.

Daily intake of 115 or 230 ml of a 40% HCl solution slightly improved calcium absorption from the gut (Hart et al., 1931). There also was increased calcium excretion in the urine resulting in a net loss of calcium in the cow. Mild acidosis induced by oral administration of ammonium chloride caused a significant increase in exchangeable calcium and a decreased accretion in bone (Vagg and Payne, 1970). These actions stimulate the calcium homeostatic mechanism and are reported to be useful in the prevention of parturient paresis. Greupner et al. (1977) showed that feeding 100 g of ammonium chloride daily for 3 weeks before parturition decreases the incidence of parturient paresis. Problems with toxicity and palatability (Horst and Jorgensen, 1972) preclude its use as a practical method to prevent parturient paresis.

Jönsson (1978) distinguishes between the preventative effect of "acid" (or "low alkalinity") diets, and diets low in pH. The amount of sodium and potassium determine the alkalinity and the amount of sulfate and chloride determine the acidity of the diet. Verdaris and Evans (1976) found that the combination of a high calcium-low pH diet increased calcium absorption, presumably by increasing the ionization of calcium in the gut. Its effectiveness in preventing parturient paresis was not reported. Dishington

(1975) found no beneficial effect of lowering pH of the diet if the alkalinity was not changed.

Short-term administration of calcium salts, particularly calcium chloride ($CaCl_2$) has been used to prevent parturient paresis. Jönsson and Pehrson (1970) gave 4 oral 150 g doses of a $CaCl_2$ gel to cows near parturition and decreased the incidence of parturient paresis from 46% in the control group to 23% in the cows receiving the $CaCl_2$ gel. This method has several disadvantages, including the unpalatability of the $CaCl_2$ and the reliance on precise calving dates.

Two current methods for preventing parturient paresis in Europe rely on the increased efficiency of absorption of calcium induced by low dietary amounts of calcium. In Britain, Pickard (1975) successfully prevented parturient paresis by limiting calcium intake to 50 g per day and phosphorus to about 30 g per day from 4-5 weeks before calving. Two or three days before calving, the intake of calcium and phosphorus were increased each by 50 g per day. There was a reduced incidence of the disease even when the additional dietary calcium and phosphorus were fed for as long as 8 days before calving. Pickard reasoned that cows adapted during the period of minimal calcium intake by increasing the efficiency of calcium absorption. Also, Pickard et al. (1975) reported that plasma PTH concentrations were increased by this treatment. In contrast, Jönsson et al. (1980) found dietary calcium intakes as low as 37 g per day did not increase plasma PTH concentrations.

Westerhuis (1974), in the Netherlands, has developed a similar dietary regime to prevent parturient paresis. Prepartal dietary calcium is kept under 50 g per day. Just after calving, an oral dose of 250 g of calcium

carbonate is given. There were no cases of the disease in 45 cows. This method has the advantage over Pickard's method in not relying on accurate prediction of calving date for its effectiveness.

Boda and Cole (1954) claimed high dietary phosphorus, when in combination with low calcium, caused a hypertrophy of the parathyroid glands and consequent stimulation of PTH secretion. In their study, the high phosphorus diet actually contained currently recommended amounts of phosphorus (National Research Council, 1978). In a subsequent study of cows fed a daily calcium intake of 11 g/day, the incidence of parturient paresis decreased as dietary phosphorus was increased from 44 to 102 g per day. Tanaka et al. (1973) have shown that kidney homogenates from rats fed high phosphorus diets had decreased 1,25-$(OH)_2D$ production compared to kidney homogenates from rats fed adequate phosphorus diets. This suggests that high phosphorus diets may suppress 1,25-$(OH)_2D$ production. Hughes et al. (1975) found increased plasma concentrations of 1,25-$(OH)_2D$ in rats fed low phosphorus diets, but Edelstein et al. (1978) found the opposite pattern. Furthermore, it appears that the stimulatory effect of phosphorus on the 1-hydroxylase enzyme activity is significant only when there is severe hypophosphatemia, and that this effect is small compared with the stimulation induced by a low calcium diet (Fraser, 1980). In conclusion, manipulation of dietary phosphorus in attempts to prevent parturient paresis have yielded conficting results.

Vitamin D Metabolism

Knowledge of the role of vitamin D in calcium and phosphorus homeostasis has expanded dramatically since the discovery that vitamin D must be metabolized to a more polar metabolite, 1,25-$(OH)_2D$, before exerting its biological effects (Holick et al., 1971; DeLuca, 1979).

Vitamin D_3 is produced in the skin via a previtamin D_3 intermediate by the photoactivation of 7-dehydrocholesterol (Holick et al., 1977). Recent evidence has been presented by DeLuca (1980) that suggests skin is a target tissue for 1,25-$(OH)_2D_3$ and may be a site of positive feedback regulation. Thus, in response to a need for calcium, 1,25-$(OH)_2D$ levels would be expected to increase and production of the vitamin D_3 precursor would be stimulated.

Vitamin D and its metabolites circulate in the blood bound to a transport protein that contains a single, high affinity, vitamin D-specific binding site (Haddad and Walgate, 1976). With normal concentrations of the binding protein in plasma, less than 10% of the available binding sites usually are occupied (Fraser, 1980).

Vitamin D is enzymatically converted to 25-OH D by a vitamin D-25-hydroxylase (25-OHase) enzyme found in mammals predominantly in the liver (Bhattacharyya and DeLuca, 1973). These investigators also found what they interpreted as a regulatory inhibition of the enzyme. Other researchers (Tucker et al., 1973) found little evidence for regulation. It is suggested that the observed changes in the 25-hydroxylation rate arise as a result of vitamin D-induced changes in enzyme affinity for substrate (Fraser, 1980). Thus, 25-OHase affinity for vitamin D would decrease as intracellular concentration of vitamin D increases.

25-Hydroxyvitamin D is 1-hydroxylated exclusively in the kidney (Fraser and Kodicek, 1970) and is regulated through a variety of factors by the need for calcium and phosphorus. Boyle et al. (1971) found the *in vitro* production of 1,25-$(OH)_2D$ to be related to the concentration of calcium in the medium. Increased plasma 1,25-$(OH)_2D$ concentrations occur in response to a low calcium diet (Omdahl and DeLuca, 1977). Removal of the parathyroid glands prevents the increase of 1,25-$(OH)_2D$ in response to a low calcium diet (Garabedian et al., 1972), indicating that calcium may act indirectly via PTH to control production of 1,25-$(OH)_2D$. Parathyroid hormone increases the 1-hydroxylase activity evidently by a cAMP-mediated mechanism (Fraser and Kodicek, 1973; Horiuchi et al., 1977).

Hypophosphatemia or low dietary phosphorus also will increase 1,25-$(OH)_2D$ levels, even in parathyroidectomized animals (Tanaka et al., 1973; Hughes et al., 1975; Steele et al., 1975). Also, Baxter and DeLuca (1976) found a low phosphorus diet to increase 1-hydroxylase activity in chickens. The stimulatory ability of the low phosphorus diet, however, was small compared to that of the low calcium diet. In contrast, Edelstein et al. (1978) found a decrease in plasma 1,25-$(OH)_2D$ concentrations in rats fed a low phosphorus diet. Thus, there may be differences among species in the ability of a low phosphorus diet to stimulate 1,25-$(OH)_2D$ production. Recent evidence suggests that low phosphorus diets fed to rats may stimulate the intestinal accumulation of already existing 1,25-$(OH)_2D$ by an unknown mechanism (Ribovich and DeLuca, 1978; Sommerville et al., 1978).

Once calcium and phosphorus needs have been met, as reflected by plasma calcium and phosphate concentrations, inhibition of the 1-hydroxylase will occur. Gray et al. (1972) showed the 1-hydroxylase to be inhibited by

1,25-$(OH)_2D$. Recent work by Omdahl et al. (1980) suggests that 1,25-$(OH)_2D$ acts by controlling the synthesis and turnover of the 1-hydroxylase, in addition to modulating the enzyme's endogenous activity. Specific binding of 1,25-$(OH)_2D$ has been demonstrated in chick parathyroid glands as well as in rat pituitary glands (Brumbaugh et al., 1975; Haussler et al., 1980). Evidence for a PTH-stimulating hormone in the pituitary gland has been presented (Latman, 1980). These recent studies suggest the possibility of feedback regulation mechanisms similar to that of many steroid hormones that involve the pituitary gland.

1,25-Dihydroxyvitamin D, the biologically active form of vitamin D, acts primarily in the intestine, bone and kidney to raise plasma levels of calcium and phosphorus. The precise mechanism of action whereby 1,25-$(OH)_2D$ stimulates intestinal absorption of calcium and phosphorus is complex and still under intense investigation. 1,25-Dihydroxyvitamin D binds to a specific cytosolic receptor in the intestine (Brumbaugh and Haussler, 1973). The hormone receptor complex then is transported to the nucleus, associates with the chromatin, and results in the *de novo* production of mRNA for calcium binding protein (CaBP) (Emtage et al., 1973; Brumbaugh and Haussler, 1974; Charles et al., 1977). The concentration of alkaline phosphatase in the intestinal brush border cells increases, and mucosal content of cAMP increases (Norman et al., 1970; Walling et al., 1976). The role that each of these three substances plays in stimulating calcium absorption from the gut is uncertain.

There is an approximate lag time of about 2 hours after injection of 1,25-$(OH)_2D$ before an increase in calcium absorption is detected (Spencer et al., 1976). Early studies showed both a direct quantitative and

qualitative relationship between the content of CaBP in the intestinal mucosa and calcium transport ability of the intestine (Wasserman and Carradino, 1973). Recently, however, 1,25-$(OH)_2D_3$-stimulated calcium transport has been found to increase before CaBP production (Spencer et al, 1976). There is a transient increase in duodenal cAMP concentrations 1/2 - 1 hour after administration of 1,25-$(OH)_2D$, coincident with a rise in DNA synthesis (Corradino, 1977). From this and other data (Zerwekh et al., 1976), Corradino (1977) suggests that stimulation of mucosal cell proliferation via a 1,25-$(OH)_2D$-stimulated, cAMP-dependent protein kinase is possible.

Alkaline phosphatase activity does not increase until about 16 hours after 1,25-$(OH)_2D$ administration (Wasserman et al., 1977). Thereafter, its activity correlates with active calcium transport. Wasserman et al. (1977) distinguishes between a dual effect of 1,25-$(OH)_2D$ on the intestinal transport of calcium, each with different time patterns: a vitamin D-dependent diffusion of calcium across the gut and an active calcium transport. Wilson and Lawson (1977) have found that two unidentified brush border membrane proteins are synthesized more rapidly than CaBP in response to 1,25-$(OH)_2D$.

In summary, the following sequence appears after administration of 1,25-$(OH)_2D$: cAMP levels and rates of DNA synthesis increase within 1 hour and might act by stimulating cell proliferation; synthesis of CaBP is stimulated and concentrations of this protein correlate well with 1,25-$(OH)_2D$-stimulated diffusional calcium transport (through 8 hr. post-injection); alkaline phosphatase activity increases (16-72 hr.) and might be involved in the active transport of calcium across an electrochemical gradient.

Intestinal transport of phosphate is stimulated by vitamin D (Harrison and Harrison, 1961). This phosphate transport mechanism is separate from calcium transport and is sodium (Na^+) dependent (Taylor, 1974). This phosphate transport mechanism also is energy dependent and involves a Na^+-K^+ATPase and related Na^+ pump (Taylor, 1974). Unlike diffusional calcium transport, diffusional transport of phosphate is not affected by vitamin D (Fuchs and Peterlik, 1980). Diffusional transport of phosphate does not require Na^+, whereas the vitamin D-stimulated phosphate transport does require Na^+. Vitamin D, presumably acting through 1,25-$(OH)_2$D, increases the maximum velocity of the transport mechanism for phosphate without any observable effect on the carrier affinity of the process (Fuchs and Peterlik, 1979; Fuchs and Peterlik, 1980). Very little else is known about the specific mechanisms of 1,25-$(OH)_2$D stimulated phosphate transport, except that it could involve the synthesis of a phosphate carrier protein (Peterlik and Wasserman, 1977).

1,25-Dihydroxyvitamin D acts, together with PTH, on bone to induce bone resorption, increase the rate of bone resorption, and increase the activity of osteoclasts (Bingham et al., 1969; Raisz et al., 1972a; Baylink et al., 1980). Specific receptors for 1,25-$(OH)_2D_3$ have been found in chick and rat bones (Kream et al., 1977). 1,25-Dihydroxyvitamin D is translocated to the nucleus of bone cells, evidently influences transcription, increases protein syntehsis, and ultimately leads to a stimulation of bone resorption (Tanaka and DeLuca, 1971; Weber et al., 1971; Raisz et al., 1972b). Thus, the mechanism of action probably involves transcription and protein synthesis, but no specific translational proteins have yet been found in response to 1,25-$(OH)_2$D.

DeLuca (1979) and others (Harrison et al., 1958; Rasmussen et al., 1963) claim that PTH is required for 1,25-$(OH)_2D_3$ (or vitamin D) to exert its function on bone. However, there is abundant evidence that each compound can act independently. Castillo et al. (1975) found 1,25-$(OH)_2D_3$ to enhance bone mineral mobilization in both intact and parathyroidectomized, phosphorus-deficient rats. Stauffer et al. (1973) found a 63% increase in bone resorption in response to a low calcium diet in vitamin D-deficient rats with high PTH levels, and a 171% increase in bone resorption in vitamin D-replete control rats. It is apparent from these studies that both PTH and 1,25-$(OH)_2D$ can act independently, and probably synergistically, to increase bone resorption.

The action of 1,25-$(OH)_2D$ in the kidney is uncertain. RNA biosynthesis and induction of CaBP in the kidney is stimulated by 1,25-$(OH)_2D$. This would indicate a functional role of 1,25-$(OH)_2D$ for this organ (Chen and DeLuca, 1973, Christakos and Norman, 1980). 1,25-Dihydroxyvitamin D stimulates renal reabsorption of calcium (Harris et al., 1976; Sutton et al., 1977). The physiological significance is questionable because, as Haussler and McCain (1977) pointed out, 99% of calcium filtered by the kidney is reabsorbed in the absence of vitamin D.

The action of 1,25-$(OH)_2D$ on tubular reabsorption of phosphate is even less certain. Popovtzer and Robinette (1975) found 25-OH D_3 effects on phosphate excretion to parallel changes in urinary cAMP excretion. These results, and other studies (Popovtzer et al., 1974), led to the suggestion that the acute effect of vitamin D metabolites on phosphate handling in rats is dependent on PTH for its expression and is mediated through cAMP.

The dietary phosphorus level might be the main controlling factor of renal handling of phosphate. Steele and DeLuca (1976) found that the renal tubule can alter markedly its capacity to transport phosphate in response to variation in the dietary supply of phosphorus. In another study, Steele et al. (1975) found vitamin D deficient animals to be capable of completely reabsorbing the filtered phosphate when fed a low phosphorus diet. It appears that the kidney can reabsorb phosphate efficiently without vitamin D, and, thus, any influence of vitamin D or its metabolites on the remal handling of phosphate is of questionable physiological value.

24,25-Dihydroxyvitamin D (24,25-$(OH)_2D$) circulates in the plasma at levels 20-50 times greater than those of 1,25-$(OH)_2D$. 24-25-Dihydroxyvitamin D is produced mainly in the kidney from 25-OH D by the 25-OH D-24-hydroxylase (24-hydroxylase) enzyme (Haussler and McCain, 1977). The kidney is not the sole site of production, as evidence for extrarenal production of 24,25-$(OH)_2D$ has been found in rats (Garabedian et al., 1974). Despite intensive investigation, no target organs have been discovered for this metabolite of unknown and limited biological activity (DeLuca, 1976). The control of the 24-hydroxylase enzyme will be discussed in subsequent sections as it relates to dietary influence on calcium and phosphorus metabolism.

Dietary Influence of Calcium and Phosphorus Metabolism

There have been few studies of vitamin D metabolism in dairy cows. Consequently, much of the following discussion will rely on data from species other than the cow and on data from *in vitro* studies. Therefore, the conclusions drawn may not hold true for the dairy cow.

Dietary phosphorus

Chicks fed an adequate or high calcium, and low phosphorus diet (0.25% phosphorus) had greater amounts of intestinal CaBP and rates of calcium absorption than did chicks fed adequate or high calcium and adequate (0.65% phosphorus) or high (1.20% phosphorus) phosphorus diets (Morrissey and Wasserman, 1971). However, low calcium (0.08% calcium) and adequate or high phosphorus diets caused similar increases in intestinal CaBP and rates of calcium absorption. Additionally, there was no additive stimulation of these parameters when both low calcium and low phosphorus diets were fed. Thus, it appears a low phosphorus diet is as effective as a low calcium diet in stimulating intestinal calcium absorption.

Low phosphorus diets fed for 3-4 weeks increased plasma 1,25-$(OH)_2D$ concentrations in rats (Tanaka et al., 1973), chicks (Sommerville et al., 1978), and pigs (Haussler et al., 1977). In the rat and pig, this increase occurs in the absence of the thyroid and parathyroid glands (Tanaka and DeLuca, 1973; Haussler et al., 1977). This increase in plasma 1,25-$(OH)_2D$ concentrations could be due to a direct stimulation of the kidney 1-hydroxylase enzyme. Increased activity of the 1-hydroxylase enzyme indeed was found in rats (Baxter and DeLuca, 1976) and pigs (Sommerville et al., 1978) fed low phosphorus diets. Plama phosphate concentration varies directly with the amount of phosphorus in the diet (Steevens et al., 1971). Thus, hypophosphatemia is associated with a low phosphorus diet (Henderson and Weakley, 1930). In the chicken, Friedlander et al. (1977) found a modest inverse correlation between 1-hydroxylase activity and plasma phosphate concentrations when the plasma phosphate concentrations were less than 8 mg/100 ml. However, these workers also found a direct correlation

between 1-hydroxylase activity and plasma phosphate concentrations when the plasma phosphate concentration was greater than 4 mg/100 ml. Thus, it seems that changes in plasma phosphate concentrations between 4 and 8 mg/100 ml would not have a significant or predictable effect on the 1-hydroxylase activity. Also, a decrease in plasma phosphate concentrations from 8 mg/100 ml to 2-4 mg/100 ml produced only a twofold increase in 1-hydroxylase activity. Montecuccoli et al. (1977) did not find an increase in the *in vitro* 1-hydroxylase activity in chicks fed a 0.3-0.4% low phosphorus diet. Sommerville et al. (1978) found a 3-fold increase in the 1-hydroxylase activity of chicks fed a 0.2% phosphorus diet. In both of these studies, chicks fed a low phosphorus diet had increased concentrations of 1,25-$(OH)_2D$ in the intestinal mucosa. Therefore, adaptation to a low phosphorus diet might involve a change at the gut mucosa leading to increased accumulation of 1,25-$(OH)_2D$ in the gut rather than, or in addition to, stimulation of the 1-hydroxylase in the kidney (Ribovich and DeLuca, 1978; Sommerville et al., 1978; Fraser, 1980).

Tanaka et al. (1973) found an inverse relationship between the amount of dietary phosphorus and the plasma concentration of 24,25-$(OH)_2D$ in rats. However, Friedlander et al. (1977) found no significant correlation between plasma phosphate concentrations and 24-hydroxylase enzyme activity, and concluded that 24,25-$(OH)_2D$ is not important in modulating responses to changes in calcium and phosphorus metabolism.

Low phosphorus diets may have a dual effect on bone: a direct effect of hypophosphatemia on bone, and an indirect effect mediated by 1,25-$(OH)_2D$. Increased number and resorptive activity of osteoclasts were found in phosphorus-deficient rats (Thompson et al., 1975). Rats had increased plasma

1,25-$(OH)_2D$ concentrations (threefold) and decreased PTH concentrations within 24 hours after initiation of an adequate calcium, low phosphorus diet (0.6% calcium, 0.04% phosphorus) compared to rats fed a control diet (Radar et al., 1979). These changes were associated with an increase in bone resorption in rats fed the low phosphorus diet, suggesting that 1,25-$(OH)_2D$, in the absence of PTH, increases both the number and resorptive activity of bone osteoclasts.

In response to phosphorus deficiency in rats, Bruin et al. (1975) found an acute inhibition of bone mineral deposition within 12 hours and an increase in bone resorption within 48 hours. *In vitro*, a decrease in medium phosphate concentration increases the rate of bone resorption (Raisz et al., 1972a). Thus, Thompson et al. (1975) suggested that the resorptive response elicited by a phosphorus deficient diet could be an effect of the diet-induced hypophosphatemia per se. Low medium phosphate concentration in *in vitro* bone cell cultures increased the ionized calcium in cytosol (Borle, 1973), which could stimulate cell proliferation (Rasmussen et al., 1972). Thus, hypophosphatemia could be indirectly responsible for the increased number of bone osteoclasts. However, Baylink et al. (1980) conclude that a low phosphorus diet acts on bone predominantly by increasing 1,25-$(OH)_2D$ concentrations. 1,25-Dihydroxyvitamin D then stimulates bone resorption by increasing both the number and activity of osteoclasts. In contrast, Sommerville et al. (1978) found no increase in bone concentrations of 1,25-$(OH)_2D$ in chicks fed a low phosphorus diet even though 1-hydroxylase activity increased 3-fold and concentration of intestinal 1,25-$(OH)_2D$ increased 2-fold.

The existence of a sensitive mechanism for phosphorus retention is suggested by the abrupt decrease in urinary phosphate excretion after initiation of a low phosphorus diet (Steele and DeLuca, 1976). This adaptation, which appears within 3 days after initiation of a low phosphorus diet (Troehler et al., 1976), seems to act independently of PTH and 1,25-$(OH)_2D$ (Steele et al., 1975; Troehler et al., 1976).

In summary, low dietary phosphorus results in hypophosphatemia. The hypophosphatemia is associated with increased accumulation of 1,25-$(OH)_2D$ in intestine, increased 1-hydroxylase activity in the kidney, and possibly a direct stimulation of osteoclast proliferation. The higher 1-hydroxylase activity results in increased 1,25-$(OH)_2D$ production. 1,25-Dihydroxyvitamin D then acts at the intestine to increase both calcium and phosphorus absorption, and acts at the bone to stimulate resorption of calcium and phosphorus by increasing osteoclast numbers and activity. Finally, there appears to be an effective mechanism to decrease phosphate excretion in the kidney that is independent of the PTH and vitamin D systems.

Dietary calcium

In many ways, low dietary calcium has similar actions to that of low dietary phosphorus on calcium and phosphorus homeostatic mechanisms, with the important exception of the effects on the PTH system. Although it appears that hypophosphatemia can increase the 1-hydroxylase activity directly, hypocalcemia evidently stimulates the enzyme's activity indirectly, via stimulation of PTH secretion (DeLuca, 1979). Rats fed a 0.01% calcium diet for 8 weeks had a 13-fold increase in plasma PTH concentrations within 6 weeks and a 10-fold increase in plasma 1,25-$(OH)_2D$ concentrations within

three weeks compared to rats fed an adequate calcium (0.6% calcium) diet (Radar et al., 1979). Horiuchi et al. (1977) showed that PTH increased 1-hydroxylase via a cAMP mediated mechanism. Fraser (1980) suggests that cAMP might directly phosphorylate, and thus activate, the cytochrome P-450 component of the 1-hydroxylase enzyme complex.

The 1-hydroxylase is influenced in different ways depending on the extra-mitochondrial concentration of ionized calcium in the kidney (Fraser, 1980). The addition of calcium to kidney mitochondrial preparations decreased the 1-hydroxylase activity by as much as 50% (Fraser and Kodicek, 1973). If kidney mitochondria are prepared with EDTA, the addition of calcium to the medium will initially increase the 1-hydroxylase activity (Horiuchi et al., 1975). Continued additions of calcium inhibited the 1-hydroxylase activity. It seems that mitochondrial calcium uptake is necessary for physiological regulation of the 1-hydroxylase, but, because extracellular calcium concentration is so sensitively maintained under normal physiological conditions, content of the mitochondrial calcium itself is not a physiologic regulatory factor (Fraser and Kodicek, 1973; Fraser, 1980).

Changes in plasma calcium concentration, acting via PTH, can increase 1-hydroxylase activity as much as 18-fold (Friedlander et al., 1977). Low calcium diets seem to increase plasma 1,25-$(OH)_2D$ concentrations more effectively than low phosphorus diets (Sommerville et al., 1978; Radar et al., 1979; DeLuca, 1979). However, as stated before, low phosphorus diets stimulate intestinal absorption of calcium as effectively as low calcium diets (Morrissey and Wasserman, 1971). Low calcium diets also will increase the phosphorus influx into gut mucosal cells (Peterlik and Wasserman, 1977).

In vitro, PTH acts directly and independently of 1,25-$(OH)_2D$ to increase the rate of bone resorption (Raisz et al., 1972a). Parathyroid hormone, in the absence of 1,25-$(OH)_2D$, had a greater stimulatory effect on the activity of bone osteoclasts than on the number of osteoclasts (Holtrop et al., 1974). Supraphysiological amounts of PTH, however, were required to elicit these responses. This led Baylink et al. (1980) to suggest that PTH action on the osteoclast cell line may require a cofactor, such as 1,25-$(OH)_2D$, that was not present in these *in vitro* systems to exert its actions under normal physiological conditions.

RNA biosynthesis in osteoclasts is stimulated within 1 hour after PTH administration, and PTH may have synergistic effects with 1,25-$(OH)_2D$ on induction on bone resorption (Bingham et al., 1969; Raisz et al., 1972b). Calcium deficient diets fed to rats increased plasma 1,25-$(OH)_2D$ and PTH concentrations and mainly resulted in increased activity of osteoclasts, with only minimal effects on the number of osteoclasts (Stauffer et al., 1973; Hughes et al., 1975). Hughes et al. (1975) also showed that stimulation of bone resorption increased as the concentrations of 1,25-$(OH)_2D$ and PTH in plasma increased.

Improved renal reabsorption of calcium occurs in response to 1,25-$(OH)_2D$ (Harris et al., 1976), and, thus, should be expected to occur in animals fed a low calcium diet.

In summary, a low calcium diet, via PTH stimulation, will result in a relatively larger stimulation of the 1-hydroxylase enzyme and subsequent greater plasma 1,25-$(OH)_2D$ concentrations than a low phosphorus diet. However, the stimulation of intestinal absorption of calcium and phosphorus seems to be similar in both low calcium and low phosphorus-fed animals,

probably because of the ability of hypophosphatemia to increase 1,25-$(OH)_2D$ localization in intestinal mucosa. Parathyroid hormone and 1,25-$(OH)_2D$ seem additive in their effects on bone, and the magnitudes of these effects are related to their plasma concentrations. Hypophosphatemia does not increase plasma PTH concentrations and, unlike its effect in the intestine, does not increase 1,25-$(OH)_2D$ localization in bone. Consequently, low calcium diets might be expected to have a greater ability to stimulate bone resorption than do low phosphorus diets. Finally, increased renal calcium retention in response to a low calcium diet is dependent on a 1,25-$(OH)_2D$-mediated mechanism, but increased renal phosphorus retention in response to a low phosphorus diet is vitamin D independent.

MATERIALS AND METHODS

Animals

Twenty Jersey cows, from the Iowa State University dairy herd, which had at least one parturition before this experiment, were assigned to 4 dietary groups. Assignment was made on the basis of milk and fat production during previous lactation, age, and previous incidence of parturient paresis (Table 1).

Prepartum Diets

During their dry period, cows were fed orchard grass hay and corn silage *ad libitum*. Thirty-two days before the expected date of calving, cows were placed in tie stalls and adjusted to their assigned diet over a 3-day period. Cows were fed one of the following experimental diets: 1) low calcium, low phosphorus (LCLP); 2) low calcium, high phosphorus (LCHP); 3) high calcium, low phosphorus (HCLP); or 4) high calcium, high phosphorus (HCHP).

The components of the basal LCLP diet are listed in Table 2. This diet provided approximately 9.5 grams of calcium and 10 grams of phosphorus daily, and meets the daily requirements of energy, crude protein, and magnesium for maintenance and pregnancy of a 450 kg cow (National Research Council, 1978). The other 3 diets were formulated by appropriately supplementing this basal LCLP diet with either calcium carbonate, monosodium phosphate, or both. The high calcium diets contained approximately 86 g of calcium (190 g of supplemental calcium carbonate). The high phosphorus diets contained approximately 82 g of phosphorus (315 g of supplemental monosodium phosphate). The diets were fed once daily from about 28 days

Table 1. Characteristics of the four groups of cows

Prepartum diet	Average age of cows at parturition (mo.)	No. of parturitions	Previous incidence of parturient paresis (no. of cases)
Low-calcium, low-phosphorus (LCLP)	53	5	2
Low-calcium, high phosphorus (LCHP)	46	5	2
High-calcium, low-phosphorus (HCLP)	52	5	1
High-calcium, high-phosphorus (HCHP)	45	5	1

[a]Based on lactation immediately before experimental diet was fed.

Incidence of parturient paresis during experiment (no. of cases)	Average time fed diet prepartum (days)	Average milk production[a] (305-day, ME, 2X) (kg)	
		Milk	Fat
0	25.2	6045	268
0	25.0	6261	235
0	27.6	5831	248
4	25.4	6246	259

prepartum through parturition. After parturition, cows were gradually changed over to a regular lactation ration consisting of alfalfa hay and a grain mix. This regular lactation ration met the recommended daily calcium and phosphorus requirements for lactating cows.

Table 2. Composition of a low-calcium, low-phosphorus diet

Feed	Dry matter intake
	(kg/day)
Corn silage	2.00
Ground corn cobs	1.00
Ground shelled corn	0.65
Molasses	0.30
Urea	0.06
Corn gluten meal	0.50
Purified corn starch	1.00
Magnesium oxide	0.012
Trace minerals mix	0.025
Total	5.547

Blood Sampling

Blood was collected into 50 ml heparinized syringes by venipuncture of the external jugular vein at 5 weeks prepartum and again just before initiation of the experimental diet. Blood samples then were taken every 3 days until 10 days prepartum, then daily samples were taken through 4 days postpartum. Samples were centrifuged within 10 minutes of collection,

and the resulting plasma frozen immediately and stored at -20°C until analysis.

Parturient Paresis Treatment

Treatment of paretic cows was begun when the cow became recumbent and consisted of intravenous infusion of 500 ml of a sterile, 23% calcium borogluconate solution (containing magnesium and 5% dextrose).

Plasma Analysis

Calcium and magnesium concentrations were determined in duplicate with atomic absorption spectrophotometry (Willis, 1960a and b). Inorganic phosphate concentrations were determined by the colorimetric assay of Fiske and Subbarow (1925) adapted for use with an Auto Analyzer (Technicon Instruments Corp., Ardsley, NY). Hydroxyproline concentrations were determined by the colorimetric assay of Tepper and DeVos (1975) adapted for use with an Auto Analyzer. 25-Hydroxyvitamin D, 1,25-$(OH)_2D$, and 24,25-$(OH)_2D$ were determined by the methods of Horst et al. (1981).

Statistical Analyses

Data were analyzed by analysis of variance techniques for a 2x2 factorial design for 9 time periods beginning with initiation of feeding experimental diets and also 9 time periods beginning 4 days before calving (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Calcium, phosphorus, hydroxyproline, and 1,25-$(OH)_2D$ concentrations of a series of plasma samples from 20 Jersey cows were measured. Concentrations of these plasma constituents are plotted as a function of the number of days on each diet (Figures 1, 3, 5, 7) and the days during the peripartal period (Figures 2, 4, 6, 8) on each diet.

There were 4 cases of parturient paresis in the cows fed the high calcium, high phosphorus diet. There were no cases of parturient paresis in cows fed any of the other 3 diets.

Plasma Calcium

Prepartal changes

Plasma calcium concentrations of cows fed low calcium diets (LCHP and HCLP) decreased from 8.8 mg/100 ml on day 0 to 8.5 mg/100 ml on day 2 (Figure 1), and plasma calcium concentrations of these cows were significantly ($p < .10+$) lower on day 2 than were the plasma calcium concentrations of cows fed high calcium diets (HCLP and HCHP). By day 8, plasma calcium concentrations in cows fed low calcium diets had returned to pre-treatment levels. From day 8 to day 23, plasma calcium concentrations were similar in all cows. Other workers have described a similar decrease in plasma calcium concentration within 3 days of initiation of feeding a low calcium diet to cows (Goings et al., 1974; Green et al., 1981). Green et al. (1981) suggest that this hypocalcemia caused by the low calcium diet is the initial signal that leads to the activation of the calcium homeostatic mechanisms in the cow.

Haussler et al. (1977) have reported that a low phosphorus diet, when fed to rats and pigs, induces hypercalcemia. In the present study, there was no significant increase in plasma calcium concentrations of cows fed low phosphorus diets, regardless of the amount of dietary calcium. In Haussler's study, the hypercalcemia was accompanied by, and was presumed to be caused by, increased plasma concentrations of 1,25-$(OH)_2D$. As will be discussed later, there were no significant increases in plasma concentrations of 1,25-$(OH)_2D$ in cows fed the LPHC diet. The increased concentrations of plasma 1,25-$(OH)_2D$ measured in cows fed the LCLP diet presumably are counteracting a mild hypocalcemia and would not be expected to lead to hypercalcemia because the diet is low in calcium.

Peripartal changes

One day postpartum, plasma calcium concentrations were significantly lower ($p < .05$) in cows fed high calcium diets compared to cows fed low calcium diets (Figure 2). Also, plasma calcium concentrations one day postpartum were significantly lower ($p < .05$) in cows fed the HCHP diet than in cows fed any of the other 3 diets (Figure 2). These results are consistent with previous findings (Goings et al., 1974; Lintner, 1977; Green et al., 1981) that prepartal low calcium diets decrease the severity of hypocalcemia soon after parturition.

Feeding prepartal low dietary phosphorus had a similar effect on plasma calcium concentrations around parturition. The cows fed the HCLP diet had significantly ($p < .10$) greater plasma calcium concentrations one day postpartum than did cows fed the HCHP diet (Figure 2).

Plasma Phosphate

Prepartal changes

Cows fed prepartal low phosphorus diets had significantly ($p < .01$) lower plasma phosphate concentrations during the entire prepartal period (Figure 3) than did cows fed high phosphorus diets. These findings are consistent with those of Barton et al. (1978) in which cows fed a diet containing 30g/day of phosphorus had an average plasma phosphate concentration of 4.9 mg/100 ml compared to an average phosphate concentration of 5.3 mg/100 ml in cows fed a diet containing 90 g/day of phosphorus. All cows in their study were fed high amounts of calcium (120 g/day).

Hypophosphatemia increased plasma 1,25-$(OH)_2D$ concentrations in rats (Hughes et al., 1975; Friedlander et al., 1977). In the present study, hypophosphatemia *per se*, induced by feeding a low phosphorus diet, when in conjunction with high dietary calcium, did not significantly increase plasma 1,25-$(OH)_2D$ concentrations in cows (Figure 7). It seems that severe hypophosphatemia (in the rat < 4 mg/100 ml) is required to stimulate 1,25-$(OH)_2D$ production (Friedlander et al., 1977; Fraser, 1980). Also, concentrations of 1,25-$(OH)_2D$ in plasma of pigs did not increase in response to a low phosphorus diet until 3 to 5 weeks after beginning the dietary treatment. Possibly, the amount of dietary phosphorus in the present study was not low enough or the diet was not fed long enough to stimulate 1,25-$(OH)_2D$ production.

Cows fed the low calcium diets tended to have greater plasma phosphate concentrations than did cows fed the high calcium diets. It is likely the greater plasma 1,25-$(OH)_2D$ concentrations in cows on the low calcium diets (Figure 7) caused increased intestinal absorption of phosphorus.

The higher plasma hydroxyproline concentrations in the cows fed the low calcium diets (Figure 5) may be indicative of increased bone resorption (Black and Capen, 1971). The phosphate liberated during bone resorption would also contribute to the plasma phosphate pool and tend to increase the plasma phosphate.

Peripartal changes

Plasma phosphate concentrations were significantly ($p < .01$) lower in cows fed low phosphorus diets from day -4 through -1 day before parturition (Figure 4). The plasma phosphate concentrations of all 4 groups of cows were similar within 2 days after they were switched to the regular lactation ration.

Plasma Hydroxyproline

Prepartal changes

Plasma hydroxyproline concentrations of cows fed low calcium diets tended to increase after initiation of the low calcium diets (Figure 5). From day 11-23, the plasma concentration of hydroxyproline of cows fed low calcium diets was significantly ($p < .10$) greater than that of cows fed high calcium diets. Plasma and urinary hydroxyproline concentrations are reported as useful indicators of bone resorption activity (Black and Capen, 1971). Other investigators have reported increases in plasma or urinary hydroxyproline concentration after feeding a low calcium diet to dairy cows (Yarrington et al., 1977; Lintner, 1977; Green et al., 1981). Thus, it seems that a low calcium diet stimulates bone resorption of calcium and phosphorus prepartum, thus activating calcium homeostatic mechanisms before parturition.

In contrast to the results of the low calcium diets, there was no indication of increased bone resorption caused by prepartal low dietary phosphorus (Figure 5). Although low phosphate concentration *per se* will stimulate bone resorption (Thompson et al., 1975; Raisz et al., 1969), this effect has only been demonstrated *in vitro*, and Baylink et al. (1980) question the physiological significance of this *in vitro* stimulation. Prepartal plasma 1,25-$(OH)_2D$ concentrations were not increased in cows fed low phosphorus diets (Figure 7). Low phosphorus diets will increase intestinal localization of 1,25-$(OH)_2D$, but they have not been shown to increase bone localization of 1,25-$(OH)_2D$ (Sommerville et al., 1978). Also, Radar et al. (1979) reported that a low phosphorus adequate calcium diet decreased plasma PTH concentrations. Because both PTH and 1,25-$(OH)_2D$ stimulate bone resorption (Raisz, 1963; Raisz et al., 1972a), it is unlikely that cows in the present study increased bone resorption in response to the low phosphorus in the diet.

Peripartal changes

Cows fed low calcium diets had significantly higher ($p < .05$) plasma hydroxyproline concentrations than did cows fed high calcium diets from -4 days through -1 days before parturition (Figure 6). These results are consistent with those of Green et al. (1981), who found significantly greater plasma hydroxyproline concentrations before parturition in cows fed low calcium, adequate phsophorus diets. The results of this study indicate that cows fed prepartal low calcium diets are actively resorbing calcium and phsophorus from bone before parturition and, thus, are able to readily respond to the calcium demands at parturition. Plasma

hydroxyproline concentrations increased in all groups of cows after parturition. This trend is similar to findings of Green et al. (1981).

Plasma 1,25-Dihydroxyvitamin D

Prepartal changes

During the entire prepartal period after initiation of the experimental diets, plasma 1,25-$(OH)_2D$ concentrations were significantly (day 2, 8, 14, 17, 20, 23: $p < .01$; day 5, 11: $p < .05$) higher in cows fed low calcium diets than those in cows fed high calcium diets (Figure 7). Efficiency of utilization of dietary calcium (percentage of calcium absorbed) is inversely related to intake (Huffman et al., 1930; Ramberg and Kronfeld, 1971). This adaptation to the amount of calcium in the diet is mediated by 1,25-$(OH)_2D$ (Omdahl and DeLuca, 1977). As shown in Figure 7, plasma 1,25-$(OH)_2D$ concentrations in cows tended to decrease after being switched to high calcium diet. High dietary intake of calcium by prepartal cows is also associated with lower PTH content in the parathyroid glands and decreased response to an EDTA challenge (Rowland et al., 1972; Black et al., 1973). Thus, cows fed high calcium diets tend to have depressed calcium homeostatic mechanisms. Green et al., (1981) suggested that the higher prepartal plasma 1,25-$(OH)_2D$ concentrations, together with increased plasma PTH concentrations, provide for effective calcium homeostasis at parturition in cows fed low calcium diets. Belyea et al. (1976) found that low calcium diets improved the ability of cows to respond to an EDTA challenge.

The amount of dietary phosphorus had no significant effect on plasma 1,25-$(OH)_2D$ concentrations, but cows fed the HCLP diet tended to have higher prepartal 1,25-$(OH)_2D$ concentrations in plasma than did cows fed

the HCHP diet. Low dietary phosphorus stimulates 1,25-$(OH)_2D$ production (Tanaka and DeLuca, 1971) and calcium absorption from the gut (Morrissey and Wasserman, 1971). Sommerville et al. (1978) showed low dietary phosphorus to increase the localization of 1,25-$(OH)_2D$ in gut mucosa without causing any measurable increase in plasma concentrations of 1,25-$(OH)_2D$. Thus, it is possible that the low dietary phosphorus in the HCLP diet can counteract the depression of plasma 1,25-$(OH)_2D$ concentrations associated with high dietary calcium intake. In this way, low dietary phosphorus may be helpful in preventing parturient paresis caused by high prepartal dietary intake of calcium.

Peripartal changes

Around the time of parturition, plasma 1,25-$(OH)_2D$ concentrations tended to increase most dramatically in cows fed the HCHP diet (Figure 8), possibly due to the severe hypocalcemia at this time. Horst et al. (1978) have shown an inverse correlation between plasma calcium concentrations and plasma 1,25-$(OH)_2D$ concentrations.

Figure 1. Plasma calcium concentrations in cows during prepartum period.

SEM for each mean value is indicated by the bar graph; data for cows fed the 4 diets are represented as indicated in the key. Refer to Materials and Methods section for explanation of symbols. Day 0 represents day of change to experimental diet, which was about 26 days prepartum. Lower case letters represent statistically significant differences (a: $P < .10$; b: $P < .05$; c: $P < .01$) in calcium concentrations for diet effects on indicated days

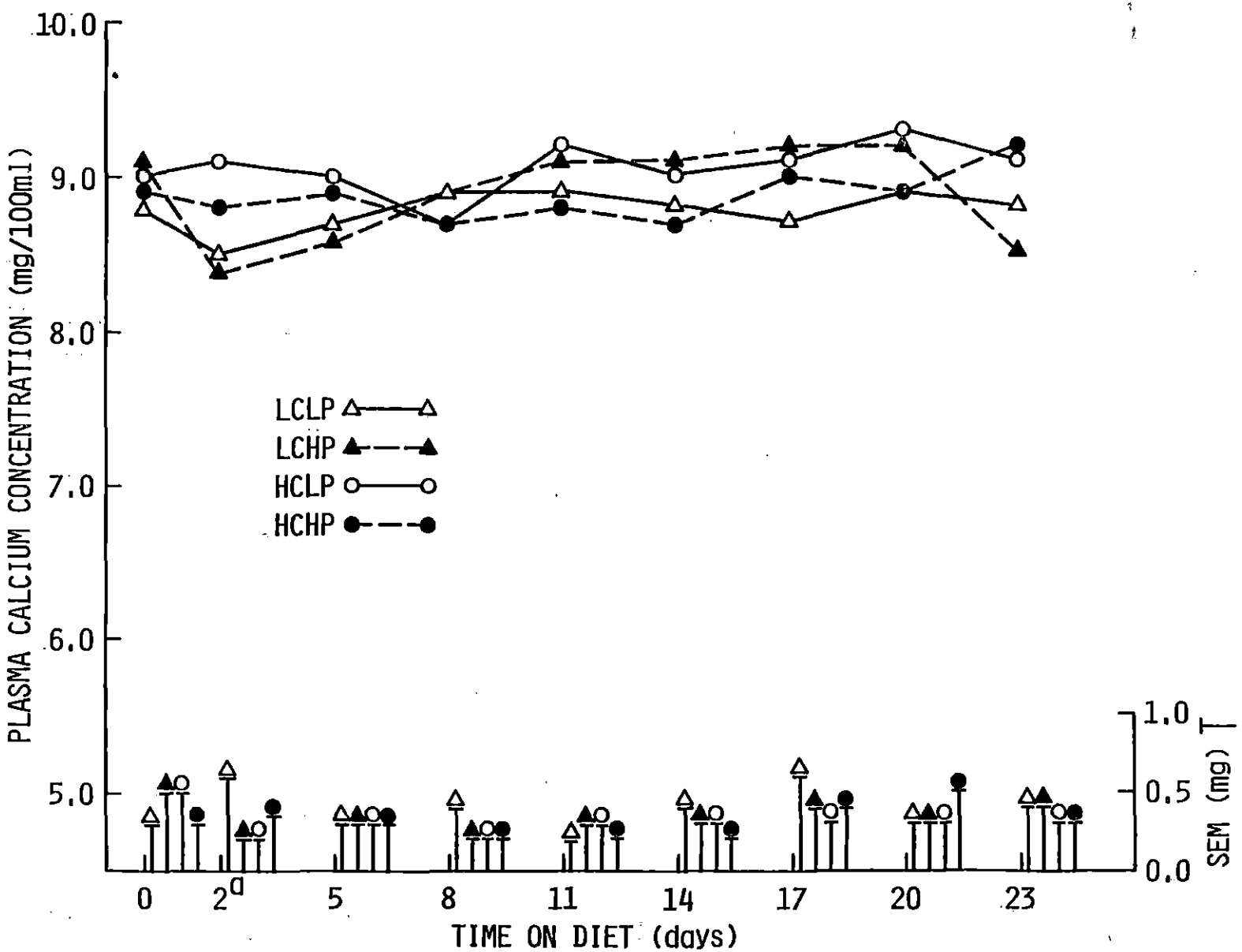

10.0
9.0
8.0
7.0
6.0
5.0
PLASMA CALCIUM CONCENTRATION (mg/100ml)
LCLP
LCHP
HCLP
HCHP
1.0
0.5
0.0
SEM (mg)
0
2d
5
8
11
14
17
20
23
TIME ON DIET (days)

Figure 2. Plasma calcium concentrations in cows during peripartal period

SEM for each mean value is indicated by the bar graph; data for cows fed the 4 diets are represented as indicated in the key. Refer to Materials and Methods section for explanation of symbols. Day 0 represents day of parturition. Lower case letters represent statistically significant differences (a: $P < .10$; b: $P < .05$; c: $P < .01$) in calcium concentrations for diet effects on indicated days

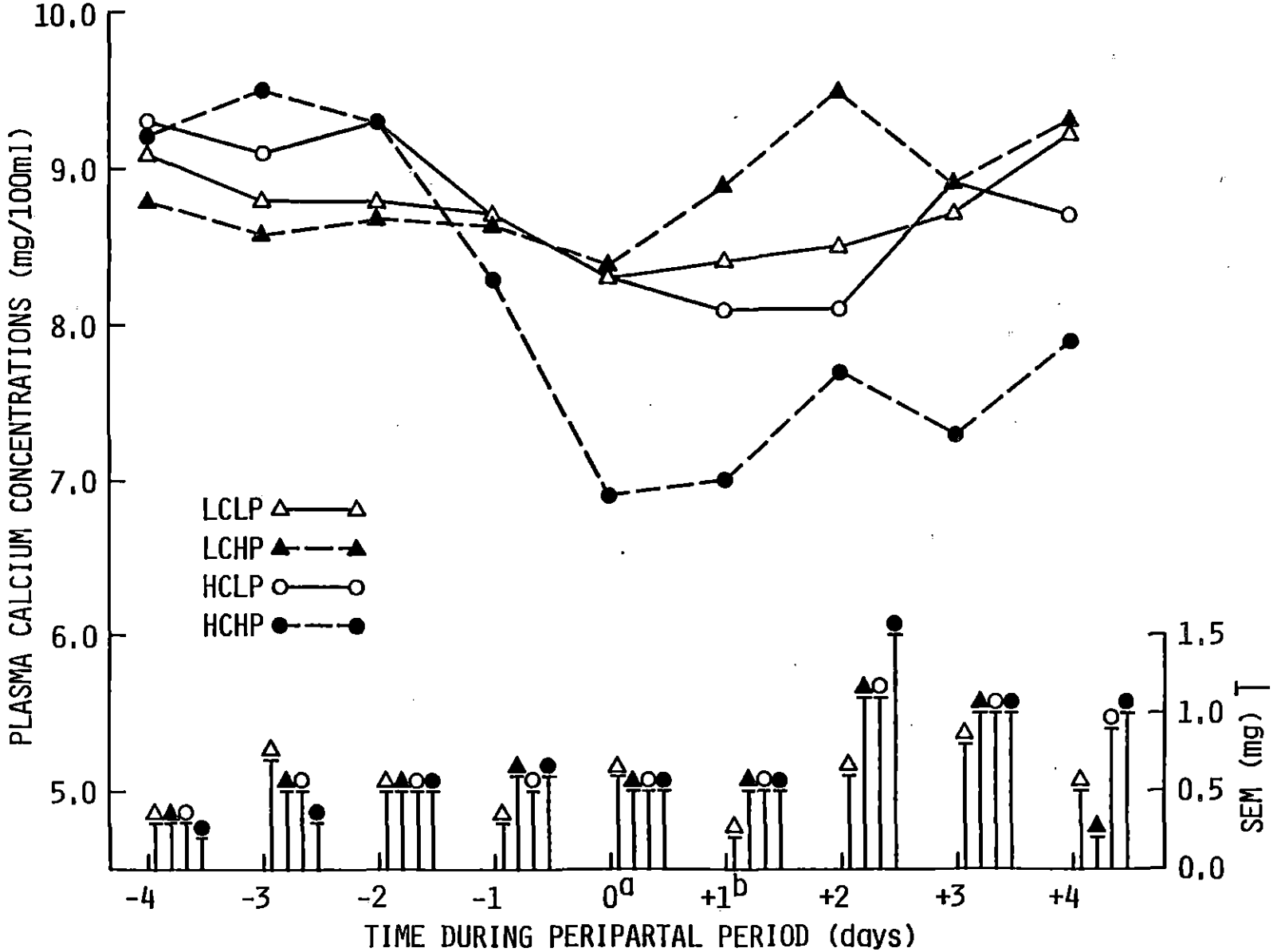

10.0
9.0
8.0
7.0
6.0
5.0
PLASMA CALCIUM CONCENTRATIONS (mg/100ml)
LCLP
LCHP
HCLP
HCHP
1.5
1.0
0.5
0.0
SEM (mg)
-4
-3
-2
-1
0a
+1b
+2
+3
+4
TIME DURING PERIPARTAL PERIOD (days)

Figure 3. Plasma phosphate concentrations in cows during prepartum period.

SEM for each mean value is indicated by the bar graph; data for cows fed the 4 diets are represented as indicated in the key. Refer to Materials and Methods section for explanation of symbols. Day 0 represents day of change to experimental diet, which was about 26 days prepartum. Lower case letters represent statistically significant differences (a: $P < .10$; b: $P < .05$; c: $P < .01$) in phosphate concentrations for diet effects on indicated days

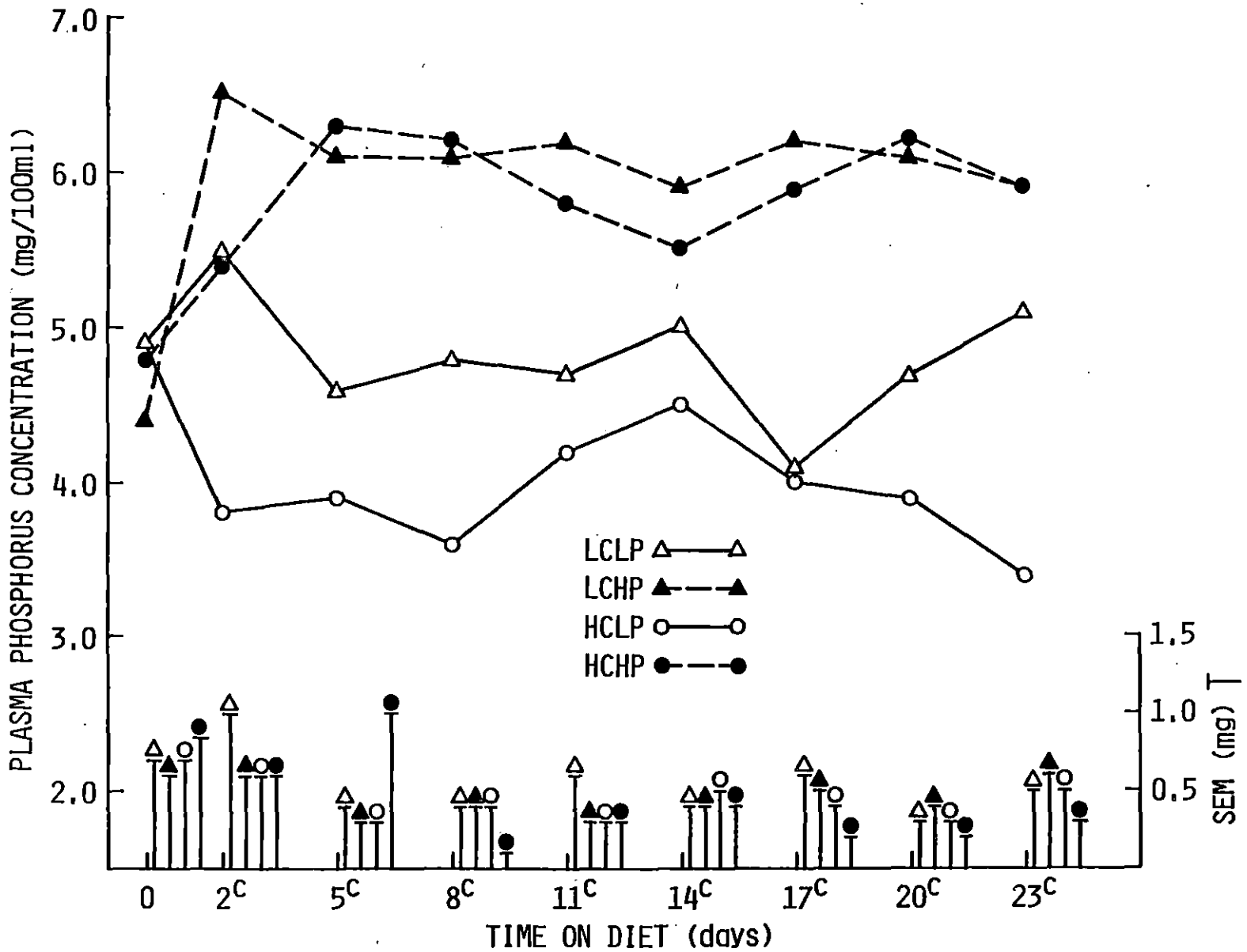

7.0
6.0
5.0
4.0
3.0
2.0
PLASMA PHOSPHORUS CONCENTRATION (mg/100ml)
LCLP
LCHP
HCLP
HCHP
1.5
1.0
0.5
SEM (mg)
0
2C
5C
8C
11C
14C
17C
20C
23C
TIME ON DIET (days)

Figure 4. Plasma phosphate concentrations in cows during peripartal period

SEM for each mean value is indicated by the bar graph. Data for cows fed the 4 diets are represented as indicated in the key. Refer to Materials and Methods section for explanation of symbols. Day 0 represents day of parturition. Lower case letters represent statistically significant differences (a: $P < .10$; b: $P < .05$; c: $P < .01$) in phosphate concentrations for diet effects on indicated days

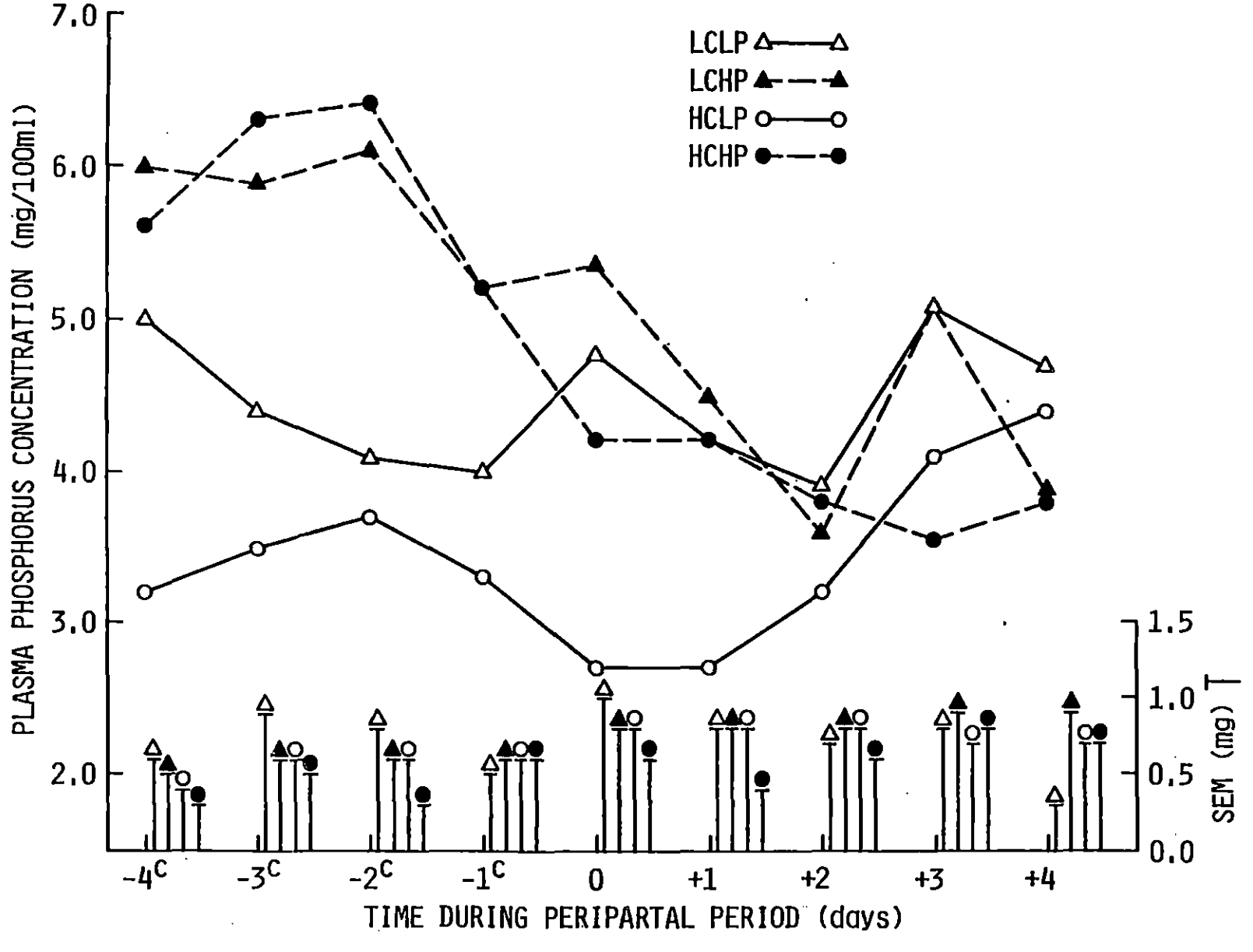

LCLP
LCHP
HCLP
HCHP
PLASMA PHOSPHORUS CONCENTRATION (mg/100ml)
7.0
6.0
5.0
4.0
3.0
2.0
1.5
1.0
0.5
0.0
SEM (mg)
-4C
-3C
-2C
-1C
0
+1
+2
+3
+4
TIME DURING PERIPARTAL PERIOD (days)

Figure 5. Plasma hydroxyproline concentrations in cows during prepartum period

SEM for each mean value is indicated by the bar graph. Data for cows fed the 4 diets are represented as indicated in the key. Refer to Materials and Methods section for explanation of symbols. Day 0 represents day of change to experimental diet, which was about 26 days prepartum. Lower case letters represent statistically significant differences (a: $P < .10$; b: $P < .05$; c: $P < .01$) in hydroxyproline concentrations for diet effects on indicated days

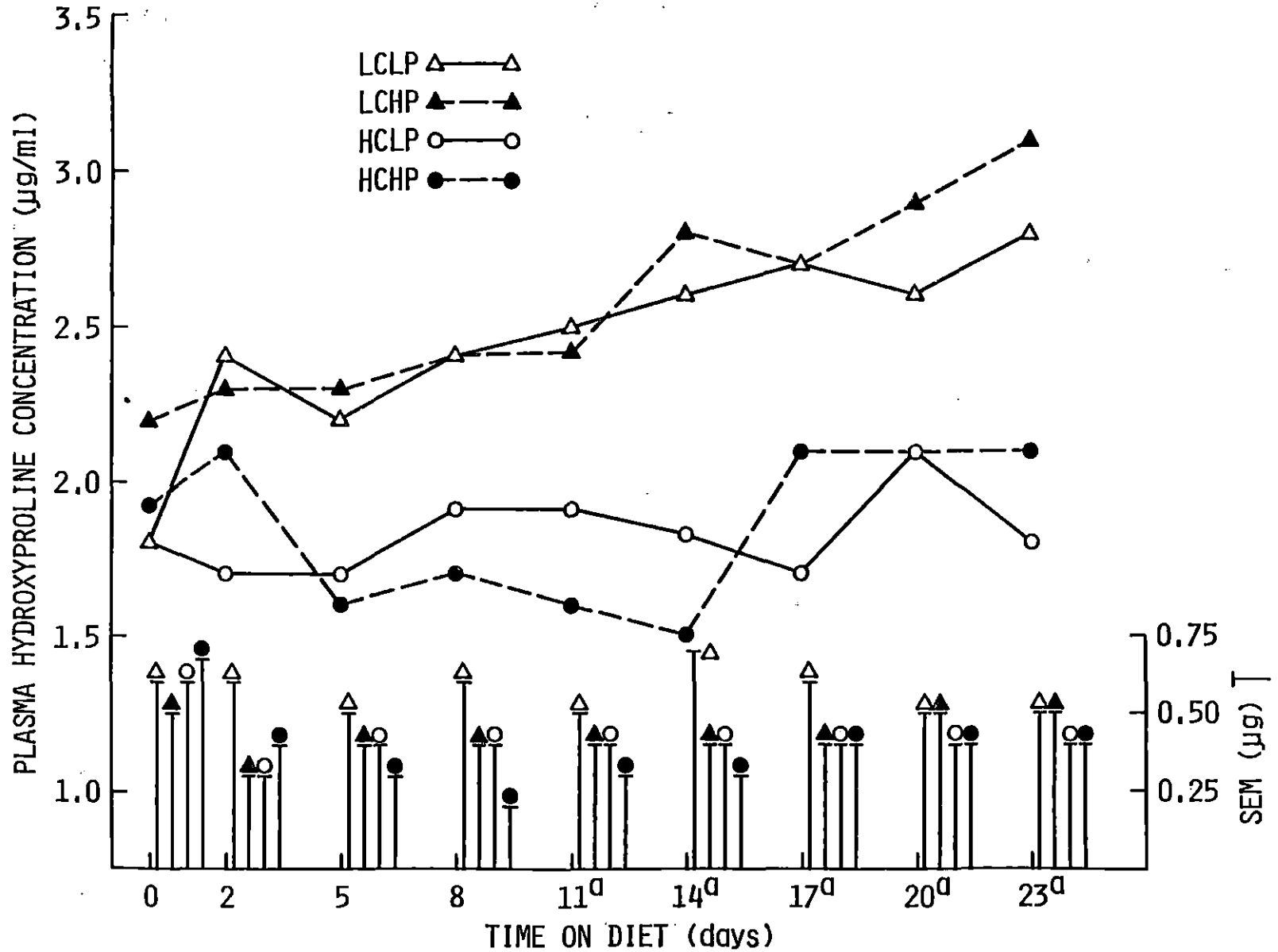

LCLP
LCHP
HCLP
HCHP
PLASMA HYDROXYPROLINE CONCENTRATION (µg/ml)
3.5
3.0
2.5
2.0
1.5
1.0
0.75
0.50
0.25
SEM (µg)
0
2
5
8
11d
14d
17d
20d
23d
TIME ON DIET (days)

Figure 6. Plasma hydroxyproline concentrations in cows during peripartal period

SEM for each mean value is indicated by the bar graph. Data for cows fed the 4 diets are represented as indicated in the key. Refer to Materials and Methods section for explanation of symbols. Day 0 represents day of parturition. Lower case letters represent statistically significant differences (a: $P < .10$; b: $P < .05$; c: $P < .01$) in hydroxyproline concentrations for diet effects on indicated days

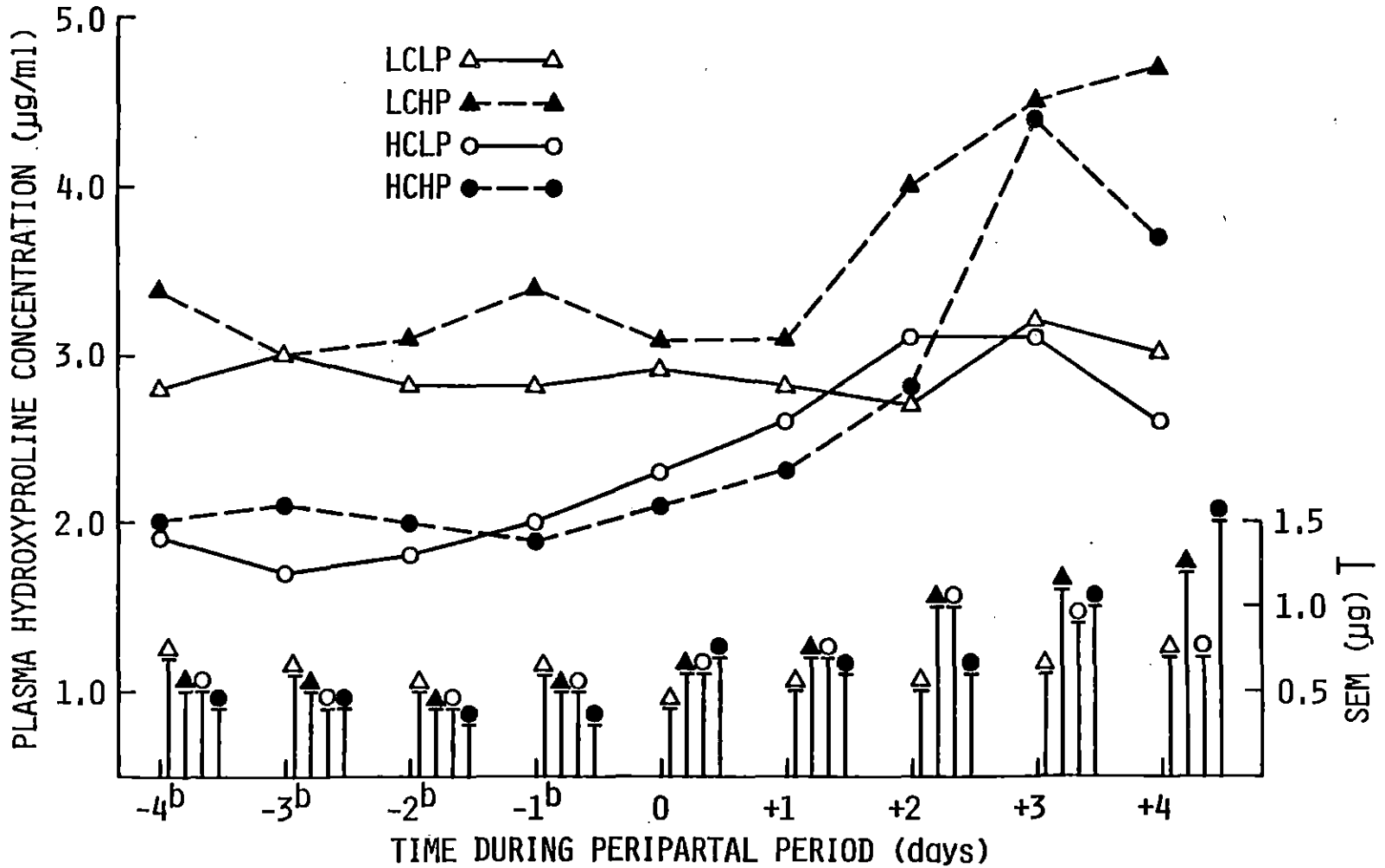

LCLP
LCHP
HCLP
HCHP
PLASMA HYDROXYPROLINE CONCENTRATION (µg/ml)
5.0
4.0
3.0
2.0
1.0
1.5
1.0
0.5
SEM (µg)
-4b
-3b
-2b
-1b
0
+1
+2
+3
+4
TIME DURING PERIPARTAL PERIOD (days)

Figure 7. Plasma 1,25-$(OH)_2D$ concentrations in cows during prepartum period

SEM for each mean value is indicated by the bar graph. Data for cows fed the 4 diets are represented as indicated in the key. Refer to Materials and Methods section for explanation of symbols. Day 0 represents day of change to experimental diet, which was about 26 days prepartum. Lower case letters represent statistically significant differences (a: $P < .10$; b: $P < .05$; c: $P < .01$) in 1,25-$(OH)_2D$ concentrations for diet effects on indicated days

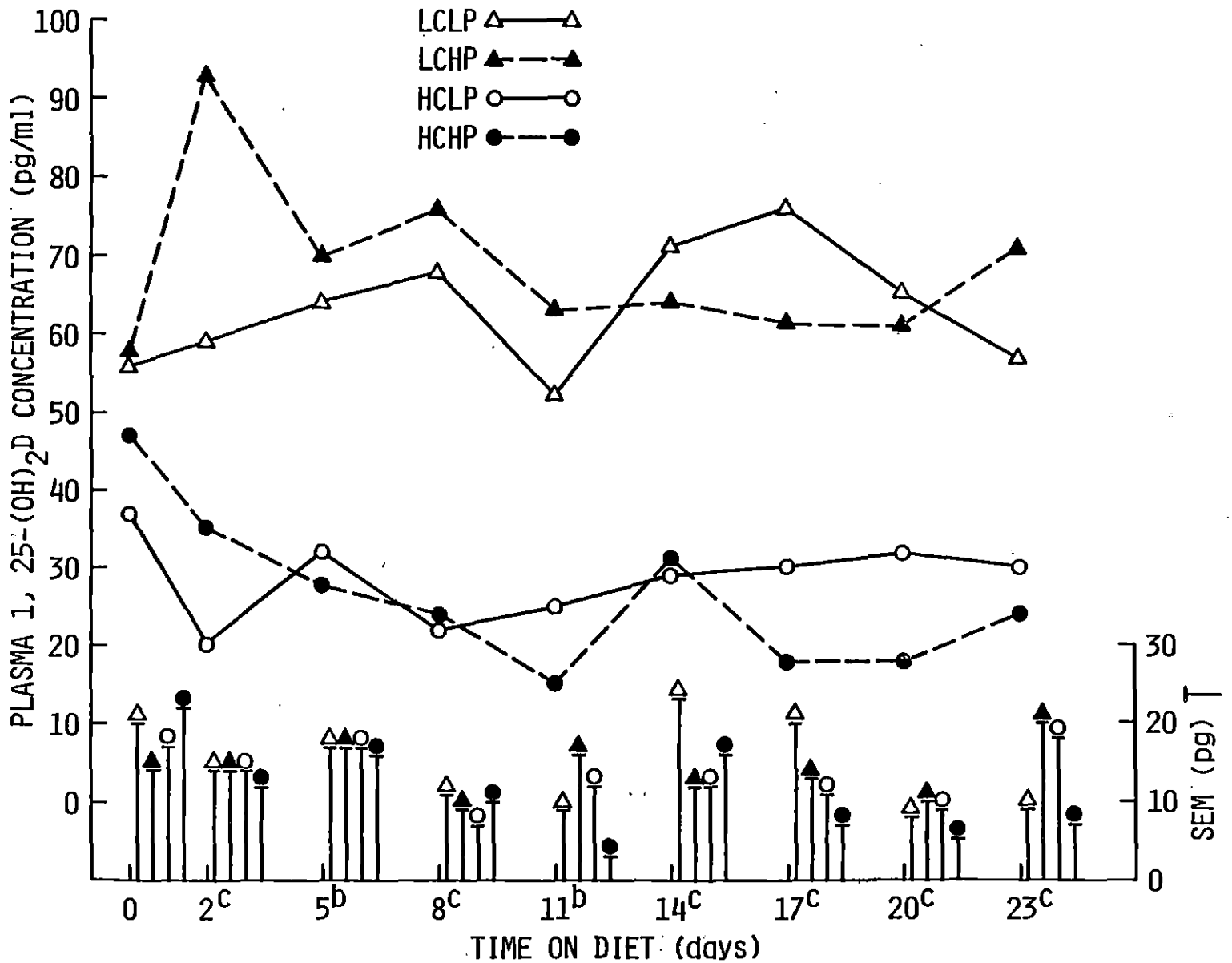
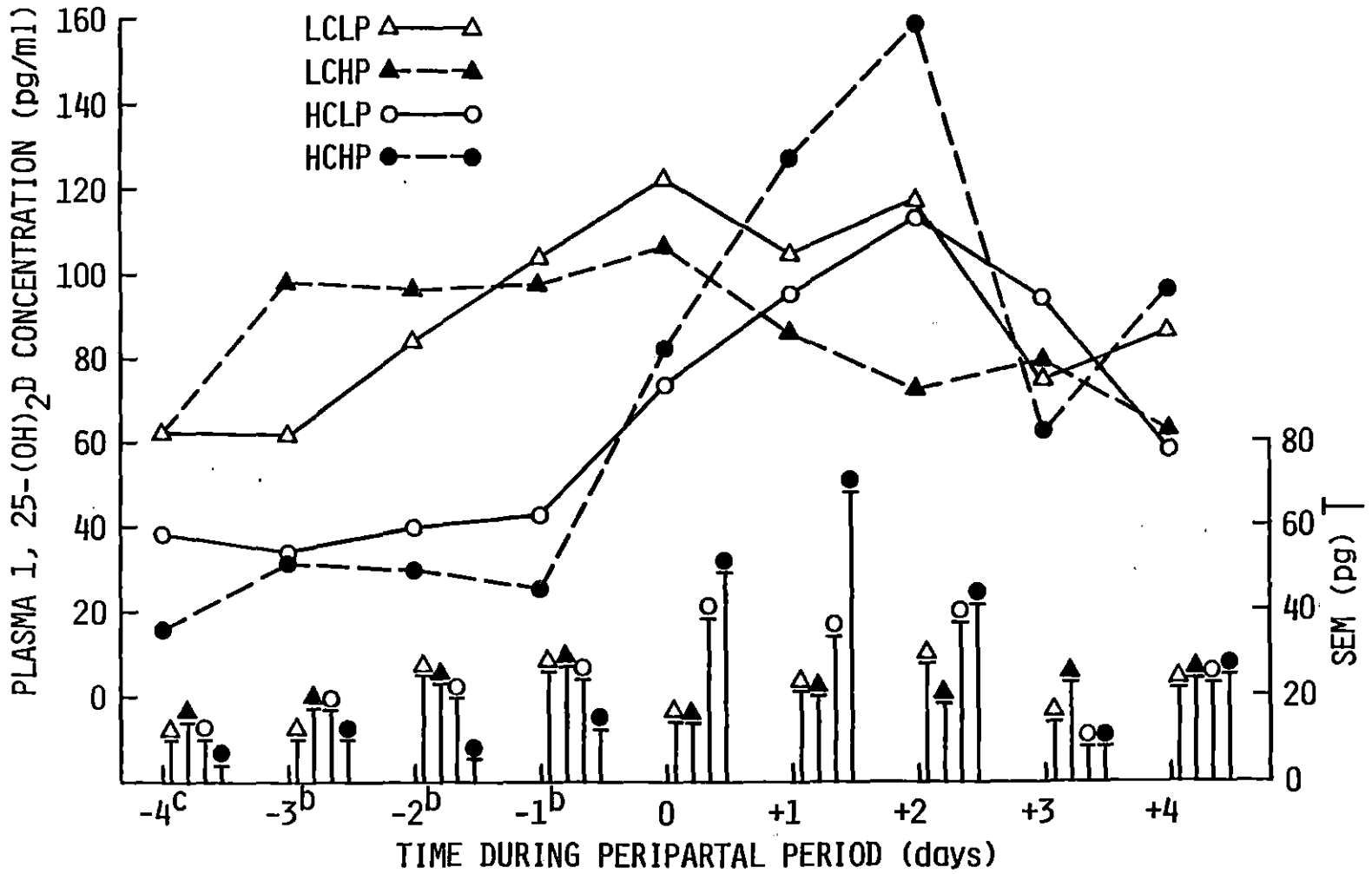

LCLP
LCHP
HCLP
HCHP
PLASMA 1, 25-(OH)2D CONCENTRATION (pg/ml)
100
90
80
70
60
50
40
30
20
10
0
30
20
10
0
SEM (pg)
0
2c
5b
8c
11b
14c
17c
20c
23c
TIME ON DIET (days)

Figure 8. Plasma 1,25-$(OH)_2D$ concentrations in cows during peripartal period

SEM for each mean value is indicated by the bar graph. Data for cows fed the 4 diets are represented as indicated in the key. Refer to Materials and Methods section for explanation of symbols. Day 0 represents day of parturition. Lower case letters represent statistically significant differences (a: $P < .10$; b: $P < .05$; c: $P < .01$) in 1,25-$(OH)_2D$ concentrations for diet effects on indicated days


LCLP
LCHP
HCLP
HCHP
PLASMA 1, 25-(OH)2D CONCENTRATION (pg/ml)
160
140
120
100
80
60
40
20
0
80
60
40
20
0
SEM (pg)
-4c
-3b
-2b
-1b
0
+1
+2
+3
+4
TIME DURING PERIPARTAL PERIOD (days)

SUMMARY

Parturient paresis can be effectively prevented by feeding a prepartum, low calcium diet (Goings et al., 1974). Recently, an investigation of the mechanism of action of a low calcium diet showed that plasma concentrations of 1,25-$(OH)_2D$ were greater in cows fed low calcium diets than in cows fed high calcium diets (Green et al., 1981). Green and co-workers suggested that the low calcium diet prevented parturient paresis by stimulating PTH and 1,25-$(OH)_2D$ production, which resulted in a "prepared" and effective gut and bone calcium homeostatic mechanism at parturition.

Because low phosphorus diets stimulate production of 1,25-$(OH)_2D$ in the chick (Sommerville et al., 1978), rat (Hughes et al., 1975), and pig (Haussler et al., 1977), it was thought that the amount of prepartal dietary phosphorus may influence vitamin D metabolism and, thus, incidence of parturient paresis in dairy cows. Thus, this study was undertaken to examine the influence of different prepartal dietary combinations of phosphorus and calcium on vitamin D metabolism and incidence of parturient paresis.

Twenty Jersey cows were fed one of 4 prepartal diets: 1) low calcium, low phosphorus (LCLP); 2) low calcium, high phosphorus (LCHP); 3) high calcium, low phosphorus (HCLP); or 4) high calcium, high phosphorus (HCHP). Diets were fed for about 4 weeks prepartum. Blood samples were taken every 3 days until 10 days prepartum, whereupon daily sampling was begun and continued until 4 days postpartum. Plasma samples were analyzed for concentrations of calcium, phosphate, magnesium, hydroxyproline, 1,25-$(OH)_2D$, and 24,25-$(OH)_2D$.

The major findings and conclusions of this study are:

1. Cows fed the low calcium diets, regardless of dietary phosphorus intake had: a) greater concentrations of plasma 1,25-$(OH)_2D$ and hydroxyproline prepartum; b) less of a decrease in plasma calcium at parturition; and c) less incidence (0 vs. 4 cases) of parturient paresis than did cows fed the high calcium, high phosphorus diet. It seemed that cows responded to a low calcium diet by increasing bone resorption of calcium and phosphorus, as indicated by the increases in plasma hydroxyproline concentrations after initiation of the diet. Because 1,25-$(OH)_2D$ stimulates gut absorption of calcium and phosphorus, the greater concentrations of 1,25-$(OH)_2D$ in cows fed low calcium diets would lead to improved prepartal efficiency of gut absorption of calcium and phosphorus. These results also suggest that prepartal low dietary phosphorus did not improve the low calcium diet's "stimulation" of the calcium homeostatic system. Conversely, high amounts of prepartal dietary phosphorus did not inhibit the low calcium diet's "stimulation" of the calcium homeostatic system.
2. Plasma phosphate concentrations reflected the amount of dietary phosphorus. Cows fed low phosphorus diets had significantly lower plasma phosphate concentrations than did cows fed high phosphorus diets. The wide fluctuation of plasma phosphate concentrations found in cows fed varying amounts of dietary phosphorus makes any sensitive control of phosphorus homeostasis unlikely.
3. When prepartal dietary calcium was high, low dietary phosphorus had no measurable effect on plasma 1,25-$(OH)_2D$ and hydroxyproline concentrations during the prepartum period. However, cows fed this diet had

greater plasma calcium concentrations at parturition than did cows fed a high calcium, high phosphorus diet. Recent investigations have shown low phosphorus diets fed to chicks increase 1,25-$(OH)_2D$ localization in the intestinal mucosa without causing any measurable increase in plasma 1,25-$(OH)_2D$ concentrations (Sommerville et al., 1978). Thus, it seems possible that the beneficial effect of low dietary phosphorus, when dietary calcium is high, may be due to a prepartal increase in efficiency of absorption of calcium and phosphorus from the gut caused by increased binding of 1,25-$(OH)_2D$ to intestinal receptors.

4. No significant differences in plasma magnesium and 24,25-$(OH)_2D$ concentrations were found in cows fed any of the four diets.

The results of this study are consistent with the following hypothesis: Low calcium diets, regardless of dietary phosphorus intake, prevent parturient paresis by activating prepartally both the bone and gut. When dietary calcium is high, low dietary phosphorus seems helpful in preventing parturient paresis by activating the gut.

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