Vitamin D metabolism

of the

neonatal calf

by

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A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Department: Veterinary Physiology and Pharmacology

Major: Veterinary Physiology

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Iowa State University
Ames, Iowa

1980

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GENERAL INTRODUCTION

Calcium homeostasis is a complex endocrinological process involving phosphorus, parathyroid hormone, calcitonin, and the vitamin D metabolites. The homeostatic mechanism is capable of meeting the calcium requirements of the animal under most conditions. However, there are certain pathological conditions in which the homeostatic mechanism is impaired. One such state is exhibited in the newborn infant - neonatal hypocalcemic tetany. The major clinical symptom of neonatal hypocalcemic tetany is the contraction of skeletal muscles in tetanic spasms and a sub-normal concentration of calcium in the plasma. Left untreated the infant would die.

Hillman and Haddad (1974) determined that one of the contributing factors in neonatal hypocalcemic tetany was the low plasma 25-hydroxyvitamin D concentration in the afflicted infants and their mothers. It was our hope to use the cow at parturition and the calf at birth as models for neonatal hypocalcemic tetany and to elucidate the calcium homeostatic mechanism of the newborn animal with respect to vitamin D metabolism.

LITERATURE REVIEW

Vitamin D has proved to be essential in the homeostasis of calcium and phosphorus. As early as 1919, Sir Edward Mellanby recognized the anti-rachitic activity of vitamin D. Later, it was shown that vitamin D expressed its anti-rachitic activity by increasing intestinal calcium absorption (Nicolaysen, 1937) and intestinal phosphate absorption (Harrison and Harrison, 1961) and by increasing the mobilization of calcium and phosphorus from bone (Harrison et al., 1958; Rasmussen et al., 1963). These processes are not only important in mineral homeostasis, but are essential to the normal remodeling of bone under various stresses. It also appears that vitamin D can increase renal tubular reabsorption of calcium (Steele et al., 1975). All of these mechanisms can be called upon to raise plasma calcium and phosphorus concentrations to ensure normal bone growth and neuromuscular activity.

Metabolism of vitamin D

The observation that there was a considerable lag time between administration of vitamin D and the initiation of its effects prompted the theory that vitamin D has to be changed in some way to gain physiological activity. The synthesis of high specific activity tritiated vitamin D_3 and new chromatographic separation and spectrophotometric techniques allowed the separation of several metabolites of vitamin D_3. The laboratory of H. F. DeLuca discovered the first of the biologically-active metabolites, 25-hydroxycholecalciferol (25-OHD_3). It has 4 times the anti-rachitic activity of vitamin D_3 (Lund and DeLuca, 1966;

Tanaka et al., 1973). A more potent metabolite was discovered soon afterward. This is the more polar metabolite, 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$], which was found to have 10 times the anti-rachitic activity of 25-OHD_3 (Holick et al., 1971; Lawson et al., 1971).

Other metabolites of vitamin D have been discovered; however, none of these metabolites have yet been shown to have any significant anti-rachitic activity. One vitamin D metabolite, 24,25-dihydroxycholecalciferol [24,25-$(OH)_2D$], is produced by the kidney (primarily) in response to normal or high blood calcium concentrations (Omdahl et al., 1972). It is widely believed that 24-hydroxylation is one of the major paths in deactivation and excretion of vitamin D metabolites (DeLuca, 1976); however, there is some evidence that 24,25-$(OH)_2D$ is essential for normal mineralization of bone in man (Rasmussen and Bordier, 1978) and in embryonic development of chicks (Henry and Norman, 1978). There is also evidence that 24,25-$(OH)_2D$ will increase intestinal absorption of calcium. Kanis et al. (1977) reported that 1 µg/day of 24,25-$(OH)_2D$ was capable of increasing intestinal absorption of ^{47}calcium in human anephric patients. This suggested that renal conversion of 24,25-$(OH)_2D$ to a more active metabolite was not essential for the biological activity.

A summary of the metabolism of vitamin D is depicted in Fig. 1.

Vitamin D generated in the skin or ingested with the diet undergoes 25-hydroxylation by a hepatic enzyme system. 25-OHD is then

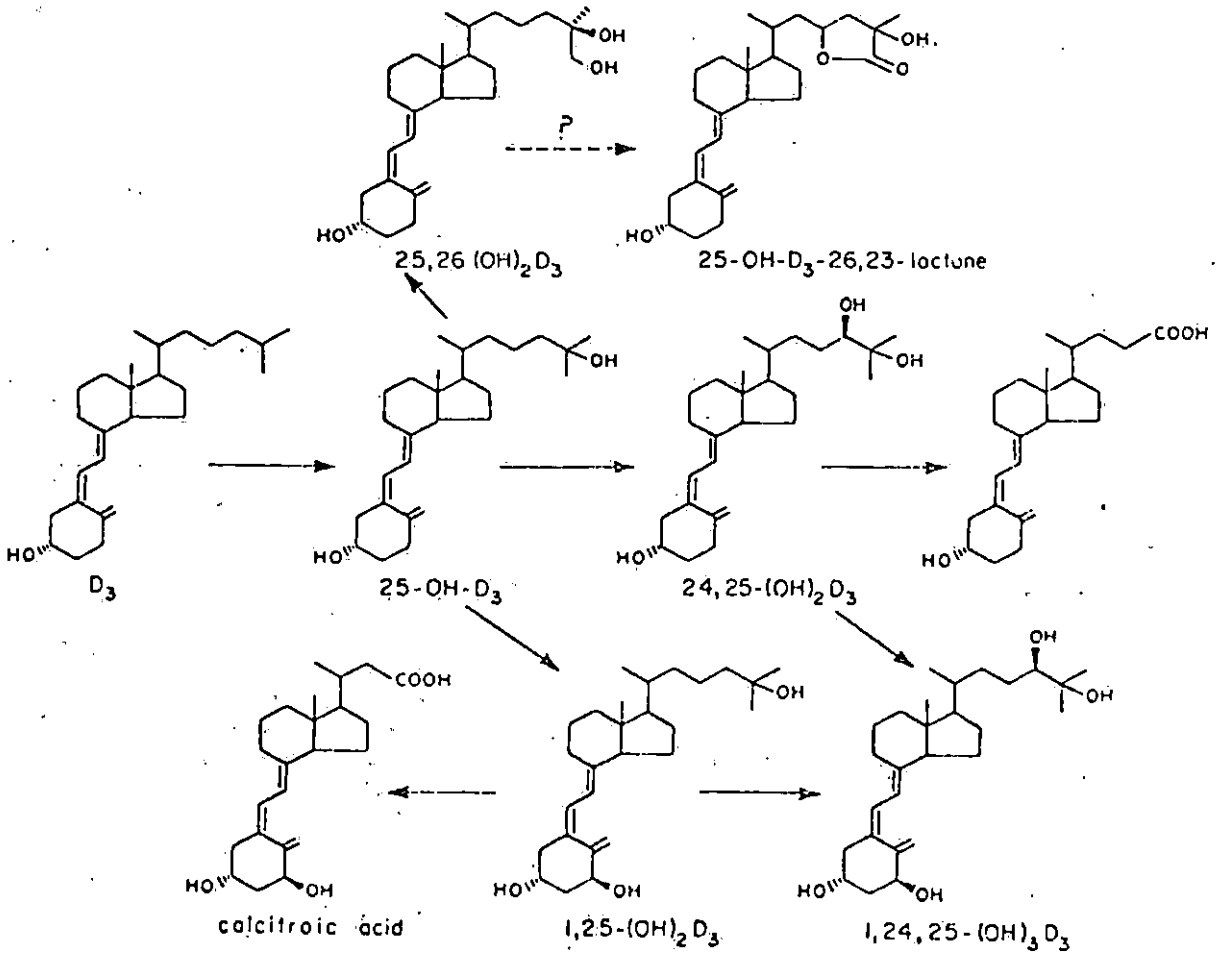

Figure 1. Metabolism of vitamin D_3 as it is currently known (DeLuca, 1979).

acted upon by a renal mitochondrial enzyme system to cause 1α-hydroxylation. The resulting metabolite, 1,25-$(OH)_2D$, is believed to be responsible for most of the anti-rachitic activities attributed to vitamin D by early workers. Proof of this arises from the fact that nephrectomized animals do not respond to vitamin D or 25-OHD, but do show increased intestinal absorption of calcium and increased mobilization of calcium from bone when treated with 1,25-$(OH)_2D$ (Boyle et al., 1972). However, 1,25-$(OH)_2D$ alone will not maintain completely normal bone remodeling (Bordier et al., 1978).

The 25-hydroxylation of vitamin D in the liver is regulated. When 25-OHD_3 is added to liver homogenates containing vitamin D_3, there is an inhibition of further 25-hydroxylation activity (Horsting and DeLuca, 1969). Also, in vivo studies show that the amount of 25-OHD_3 in the blood of animals is not proportional to the dose of vitamin D. At low doses, a high proportion of the vitamin D is converted to 25-OHD_3 (Boyle et al., 1971; Omdahl and DeLuca, 1973). It is uncertain whether the production of 25-OHD_3 inhibits the 25-hydroxylase enzyme directly or if it acts indirectly through other humoral mechanisms. This regulatory mechanism may be important in protecting against hypervitaminosis D. 25-Hydroxylase activity of the liver does not seem to change in response to changing physiological states, especially hypocalcemia and hypophosphatemia (DeLuca, 1979).

Much of the current research has centered around the renal mitochondrial 25-hydroxy-D_3-1α-hydroxylase enzyme. Boyle et al. (1971)

demonstrated that as dietary calcium decreased there was an increase in circulating 1,25-$(OH)_2D_3$. They noted that little 1,25-$(OH)_2D_3$ was produced and that the 1α-hydroxylase system was suppressed in animals on high calcium diets. There was a corresponding increase in plasma concentrations of 24,25-$(OH)_2D_3$ indicating a switch from 1α-hydroxylase activity to 24-hydroxylase activity within the kidney. Plasma calcium appeared to be controlling the production of 1,25-$(OH)_2D_3$. The plasma calcium concentration was thought to be either exerting a direct effect on the renal 1α-hydroxylase system or causing release of hormones which were affecting the activity of the 1α-hydroxylase enzyme.

Plasma parathyroid hormone concentration increases in response to falling blood calcium concentrations, and parathyroid hormone action then tends to bring the plasma calcium concentrations back to normal (Rasmussen et al., 1963). Garabedian et al. (1972) showed that removal of the parathyroid glands of rats leads to disappearance of the 1α-hydroxylase activity and appearance of the 24-hydroxylase activity. Administration of parathyroid hormone to these animals restored the 1α-hydroxylase activity of the renal mitochondria.

Thus, the parathyroid gland releases parathyroid hormone in response to the decrease in plasma calcium, and the parathyroid hormone then acts on the kidney to activate the 1α-hydroxylase system and inhibits the 24-hydroxylase system. In this way, 1,25-$(OH)_2D_3$ is produced in response to low plasma calcium and can increase plasma

calcium concentrations to normal through its hypercalcemic activities on intestine, bone, and renal tubular reabsorption.

Calcitonin is a thyroid hormone produced in response to hypercalcemia or hypermagnesemia (Littledike and Arnaud, 1971). Early reports on the effect of calcitonin on 1,25-$(OH)_2D_3$ production were confusing. It had been noted that calcitonin injection caused an increase in 1,25-$(OH)_2D_3$ production. Most of these effects could be attributed to the hypocalcemic action of calcitonin which stimulated parathyroid hormone production. Lorenc et al. (1977) concluded that calcitonin has no effect on vitamin D metabolism in thyroparathyroidectomized rats.

There is little evidence for a direct effect of calcium on the renal mitochondrial hydroxylase systems (Omdahl and Evan, 1977). Addition of calcium to mitochondrial preparations of chick kidneys does not reduce the 1α-hydroxylase activity until the calcium concentration exceeds 200 mM. At this excessive level, calcium uncouples the oxidative phosphorylation reactions within the mitochondria. At physiological concentrations, calcium has no direct inhibitory effect on the 1α-hydroxylase.

Plasma phosphorus concentration plays an important role in the regulation of vitamin D metabolism (Bacter and DeLuca, 1976). Rats on a high calcium, low phosphorus diet had increased plasma 1,25-$(OH)_2D_3$ concentrations and hypercalcemia (Tanaka and DeLuca, 1973). This response is not mediated by parathyroid hormone as phosphate depletion will lead to hypercalcemia even in thyroparathyroidectomized rats

(Hughes et al., 1976). Larkins and his co-workers (1974) reported that phosphorus concentration was involved in intracellular induction and suppression of 1α-hydroxylase and 24-hydroxylase activity. Rader et al. (1979) showed that either hypocalcemia or hypophosphatemia will stimulate production of $1,25\text{-}(OH)_2D_3$. In both cases, there was mobilization of bone mineral. However, in the event of hypocalcemia the increased rise in $1,25\text{-}(OH)_2D_3$ production is due to the increased stimulation of parathyroid hormone. The increased $1,25\text{-}(OH)_2D$ associated with hypophosphatemia seems to be attributable to removal of the inhibition of the 1α-hydroxylase present at normal plasma phosphorus concentrations.

The presence of cytosolic $1,25\text{-}(OH)_2D$ receptors within the parathyroid gland suggests the possibility that $1,25\text{-}(OH)_2D$ may control its own production through a negative feedback mechanism (Brumbaugh et al., 1975).

Action of $1,25\text{-}(OH)_2D$ on the intestine, bone, and kidney

Very little is known about the mode of action of $1,25\text{-}(OH)_2D$ at the molecular level. Calcium is transported across the intestinal epithelium against a concentration gradient (active transport) if the animal is producing $1,25\text{-}(OH)_2D$ (DeLuca, 1979). $1,25\text{-}(OH)_2D$ appears in the nuclei of intestinal crypt and villus cells prior to the initiation of intestinal calcium transport (Zile et al., 1978). Cytosolic receptors have been described for $1,25\text{-}(OH)_2D$ indicating that it may

act via a similar mechanism to that postulated for other steroid hormones, e.g. estrogen. The cytosolic receptor binds the 1,25-$(OH)_2D$, and the receptor-1,25-$(OH)_2D$ complex then moves to the nucleus (Chen and DeLuca, 1973). In vitro experiments suggest that the receptor-1,25-$(OH)_2D$ complex then binds to nuclear chromatin material (Brumbaugh and Haussler, 1974). This interaction is thought to result in production of mRNA that codes for production of specific calcium and phosphate transport proteins. Wasserman and Taylor (1966) discovered a calcium-binding protein in the intestine of chicks that was vitamin D-dependent. There is a very good correlation between the calcium transport across the intestine and the amount of calcium-binding protein. However, time course studies reveal that increased calcium transport occurs before significant calcium-binding protein synthesis occurs (Ebel et al., 1969). Also, treatment of chicks with actinomycin D - an inhibitor of messenger RNA formation - does not block the increase in intestinal calcium transport in response to 1,25-$(OH)_2D$ (Tanaka et al., 1971). Thus, it appears that 1,25-$(OH)_2D$ can increase calcium transport via uncharacterized mechanisms that are not mediated by the intestinal mucosa cell nuclei. It is known that 1,25-$(OH)_2D$ administration increases the amount of alkaline phosphatase, calcium-dependent ATPase, and actin in the intestinal mucosal cell and brush border. All have been suggested as having possible roles in calcium transport (DeLuca, 1979).

Even less is known about intestinal phosphate transport than calcium transport, though it is also an active transport process (Walling et al., 1977). No phosphate-binding protein has yet been found. Thus, the exact role of 1,25-$(OH)_2D$ in intestinal calcium and phosphate transport remains to be elucidated, though it is clear that little active transport of either calcium or phosphorus occurs in the absence of 1,25-$(OH)_2D$.

Resorption of bone is dependent on the presence of 1,25-$(OH)_2D$ (Arnaud, 1978). Tritiated 1,25-$(OH)_2D$ has been localized inside of the nucleus of osteoblasts, osteocytes, and chondrocytes, indicating binding of 1,25-$(OH)_2D$ to a cytosolic receptor and subsequent transport to the nucleus. A nuclear mechanism is likely since actinomycin D will block the 1,25-$(OH)_2D$ mediated resorption of calcium from bone, though the identity of the gene products involved remains unresolved (Tanaka and DeLuca, 1971).

Evidence that 1,25-$(OH)_2D$ acts to improve renal reabsorption of calcium and phosphorus is quite recent. Thus, little is known about the mechanism of action of 1,25-$(OH)_2D$ on the kidney.

Calcium Homeostasis of the Fetus and the Neonate

Complex changes in fetal and maternal calcium metabolism occur during pregnancy. The developing fetus requires the translocation of large amounts of calcium and phosphorus from the maternal circulation

across the placenta. The precise role vitamin D metabolism of the dam and/or feto-placental unit plays in this process is unknown.

The neonate represents another complex period of adjustment in maintenance of calcium homeostasis. Since the placenta no longer supplies calcium and phosphorus, the neonate must rely on intestinal absorption of calcium from milk if the neonate is to adjust to life outside the uterus. Some neonates do not make the transition successfully, as in the case of infant neonatal hypocalcemic tetany. Vitamin D metabolism may be important in the adjustment of the neonate to extra-uterine life (Hillman et al., 1979).

Placental Transfer

Calcium

During the last portion of gestation there is a marked fetal-maternal gradient of calcium. Thus, calcium has to be transported across the placenta against a concentration gradient (Malan, 1928; Garel and Pic, 1972; Barlet et al., 1978). The plasma calcium concentration of the fetus is independent of plasma calcium of the maternal circulation. Bawden and Wolkoff (1967) infused calcium into the circulation of pregnant ewes and detected no change in fetal plasma calcium. Barlet et al. (1979) have observed the same phenomena in cows and also have shown that hypocalcemia caused by parturient paresis in the cow

has no effect on plasma calcium of the fetal calf (Barlet et al., 1977). However, these studies involved only transient changes in blood calcium concentration of the dam. Early work on rickets and fetal mineralization showed that long-term calcium and phosphorus deficiency of the mother resulted in neonates with lower blood calcium and phosphorus (Sontag et al., 1936).

Phosphorus

Phosphorus is also transferred across the placenta against a concentration gradient. Before term, plasma phosphorus is higher in the fetus than in the mother in rodents, ruminants, and humans (Barlet et al., 1978). This indicates that phosphate transfer across the placenta is an active process, but little is known of factors affecting phosphate transfer.

Vitamin D and Its Metabolites

Neonatal rickets had long been associated with osteomalacia of the mother (Maxwell, 1935). Korenchevsky and Carr (1923) noted that vitamin D supplementation of pregnant rats delayed the time of onset of rickets in their pups placed on rachitic diets at weaning. These observations suggest that some antirachitic activity is able to cross the placenta into the fetus, though there is one report that suggests that pregnancy goes on normally with apparently normal fetuses in vitamin D-deficient rats (Halloran and DeLuca, 1979).

Haddad et al. (1971) injected tritium-labeled vitamin D_3 and tritium-labeled 25-OHD_3 into pregnant rats. Silicic acid chromatograms of lipid-soluble extracts of the whole fetuses showed the existence of tritiated vitamin D_3 and 25-OHD_3 in the fetus.

Ross et al. (1979) have confirmed that vitamin D_3 and 25-OHD_3 will cross the placenta in the sheep. In addition, they showed that administration of tritiated 1,25-$(OH)_2D_3$ to the ewe resulted in a rapid buildup of tritiated 1,25-$(OH)_2D_3$ in the fetal lamb's plasma. When the ewe was injected with tritiated vitamin D_3 or 25-OHD_3, tritiated 24,25-$(OH)_2D_3$ could be recovered from the fetal plasma as well. This indicates that this metabolite may also cross the placenta, although the possibility that the 24,25-$(OH)_2D_3$ found in fetal plasma arose from fetal renal 24-hydroxylase activity was not ruled out. It is interesting to note that these workers found that the maternal:fetal plasma concentration ratios for tritiated D_3 and tritiated 25-OHD_3 were similar (about 23:1) suggesting comparable transfer rates across the placenta. In the case of the dihydroxylated metabolites, 24,25-$(OH)_2D$ and 1,25-$(OH)_2D$, the maternal:fetal ratio was close to unity, suggesting a more rapid equilibration of these metabolites between maternal and fetal plasma compartments.

Correlations Between Vitamin D and Its Metabolites in the Maternal and the Neonatal Circulation

Vitamin D

There are no reports of vitamin D concentrations in maternal and fetal plasma of any species. Because the liver rapidly clears vitamin D from the blood, vitamin D is often undetectable in plasma. Therefore, plasma 25-OHD concentration is thought to be a better indication of the vitamin D status of animals.

25-OHD

Many studies have been done in which 25-OHD levels of the mother and neonate have been measured. Barlet et al. (1978) report a significant correlation between maternal and neonatal levels of 25-OHD in the sheep. They also report that the plasma 25-OHD level of the dam was always higher than in the neonate, suggesting passive diffusion across the placenta. They showed that the plasma concentration of 25-OHD in the fetus rises during the last month of gestation and that there was a subsequent decline for several days after birth.

Ross et al. (1976) also monitored fetal and neonatal 25-OHD levels in the sheep. They also found the rise in 25-OHD during the last portion of gestation and the high correlation between maternal and neonatal concentrations of 25-OHD. However, in contrast to the work of Barlet et al. (1978), they did not find a significant change in mean plasma

25-OHD between birth and six days postpartum. Ross et al. found that the change in plasma 25-OHD following birth was related to the original birth concentration. Those lambs which had the highest 25-OHD concentration showed a decrease by the second day postpartum, whereas those lambs which had low 25-OHD levels showed an increase by the second day postpartum. Thus, several days postpartum similar plasma 25-OHD concentrations were seen in all lambs. The neonatal levels of 25-OHD reported by Barlet's group averaged 31 ± 4.8 ng/ml plasma, whereas those studied by Ross et al. averaged 17.8 ± 3.3 ng/ml plasma. The higher levels of 25-OHD found in Barlet's lambs and the subsequent decline in plasma 25-OHD is consistent with the decline in plasma 25-OHD described by Ross et al. in those lambs born with high plasma 25-OHD concentrations.

Hillman and Haddad (1974) measured 25-OHD in maternal and infant cord blood and demonstrated a significant correlation between maternal and neonatal levels of 25-OHD. Wolf et al. (1977) studied 25-OHD levels in mothers and newborn infants at various seasons of the year. Mothers and the infants born to them in autumn had the highest 25-OHD concentrations. Conversely, infants born in winter (January-February) had the lowest circulating concentrations of 25-OHD, presumably due to lack of exposure of the mother to solar ultraviolet irradiation. Infants born with high 25-OHD levels showed a decline in 25-OHD over the first 12 days of life, whereas those born with low levels of 25-OHD tended to rise over the first 12 days.

Mendelsohn and Haddad (1975) studied the 25-OHD profiles of neonatal rats. They reported a significant decrease in 25-OHD levels, from 5-6 ng/ml plasma at birth to 1-2 ng/ml plasma by age 12 days. By 3-4 weeks of age, plasma 25-OHD concentrations had risen to 7.6-9.3 ng/ml. The rate of turnover of 25-OHD was similar at birth and at 12 days of age as indicated by the plasma disappearance rate for tritium following intraperitoneal injection of [^{3}H]-25-OHD.

Dihydroxylated metabolites

The interrelationship between 1,25-$(OH)_2$D in the mother and fetus has been only recently studied. Steichen et al. (1980) could not demonstrate any correlation between maternal 1,25-$(OH)_2$D and neonatal infant cord blood at parturition. They reported very low levels of 1,25-$(OH)_2$D in infant cord serum (19 ± 4 pg/ml). The level of 1,25-$(OH)_2$D rose during the first 24 hours of life to 31 ± 6 pg/ml, which was near the normal adult value of 29 ± 2 pg/ml. The mother's 1,25-$(OH)_2$D concentration in serum at parturition was very high, averaging 63 ± 11 pg/ml. The lack of correlation between maternal and neonatal levels of 1,25-$(OH)_2$D strongly suggests an independent control of 1,25-$(OH)_2$D by the fetus.

No correlation between 24,25-$(OH)_2$D concentrations in maternal and cord sera was demonstrated by Hillman et al. (1978).

Regulation of Vitamin D Metabolism of the Fetus and Neonate

Vitamin D metabolism is presently recognized to be regulated by the concentrations of calcium, phosphorus, and parathyroid hormone in the blood. The fetal and maternal compartments differ markedly in the concentrations of these factors in the blood, primarily due to active transport of calcium and phosphorus across the placenta. The net result is that the maternal blood compartment is relatively low in calcium and phosphorus (due to fetal drain of these elements) and normal or high in parathyroid hormone concentration (Cushard et al., 1972). In contrast, the fetus is well supplied with calcium and phosphorus and cord serum from infants shows a low level of parathyroid hormone (Hillman et al., 1978).

As a result of these differences, the metabolism of vitamin D in the mother near parturition tends toward production of $1,25\text{-}(OH)_2D$ while in the fetus, $24,25\text{-}(OH)_2D$ seems to be the major dihydroxylated form. Lester et al. (1978) injected tritiated 25-OHD into pregnant vitamin D-deficient rats on the 19th and 20th day of pregnancy. They found that fetal plasma contained 320% more $24,25\text{-}(OH)_2D_3$ than the corresponding maternal plasma. On the other hand, maternal plasma contained 360% more $1,25\text{-}(OH)_2D_3$ than the corresponding fetal plasma. In those pregnant rats nephrectomized prior to the injection of tritiated $25\text{-}OHD_3$, there was a significant reduction in the maternal plasma concentration of both $1,25\text{-}(OH)_2D$ and $24,25\text{-}(OH)_2D$. However,

there was no significant change in the concentration of either 1,25-$(OH)_2D_3$ or 24,25-$(OH)_2D_3$ in the fetal circulation. The maintenance of fetal plasma levels of 1,25-$(OH)_2D$ and 24,25-$(OH)_2D$ after maternal nephrectomy suggests that the feto-placental unit is exerting some degree of independent control on the metabolism of 25-OHD.

There is increasing evidence that prolactin, growth hormone, and other pituitary factors may play a role in regulating vitamin D metabolism (Spanos et al., 1978). Pregnancy brings about many changes in the concentrations of these factors, and the effects on vitamin D metabolism have yet to be determined.

A complicating factor in maternal-fetal vitamin D metabolism is the placenta. Weisman et al. (1978) reported that pregnant rats continue to synthesize 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ following nephrectomy. It has since been shown that 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ can be produced from 25-OHD_3 by human (Weisman et al., 1979) and rat (Tanaka et al., 1979) placental tissues in vitro. It is possible that the placenta is responsible in part for the 24,25-$(OH)_2D$ of the fetus at the end of parturition. It may also act as a protective mechanism by hydroxylating 1,25-$(OH)_2D$ at the 24-carbon position, thus reducing its hypercalcemic activity. The placenta may also be contributing to the maternal 1,25-$(OH)_2D$ pool which increases toward the end of gestation.

There is a possibility that the 1,25-$(OH)_2D$ produced by the placenta may act to regulate the active transport of calcium and phosphorus across

the placenta. Bruns et al. (1978) have demonstrated the existence of a placental calcium-binding protein similar to rat intestinal vitamin D-dependent calcium-binding protein to support this proposal.

Neonatal Adaptation to Extra-Uterine Life

Once the umbilicus has been severed or the egg shell broken, the neonatal animal can no longer rely on the placenta or the magma of the egg to supply its calcium and phosphorus requirements. For instance, in the chick these elements must be obtained from grain, whereas mammalian neonates rely on milk.

In those mammals studied there seems to be a decrease in plasma calcium and phosphorus which leads to an increase in parathyroid hormone, $1,25\text{-}(OH)_2D$, and a decrease in $24,25\text{-}(OH)_2D$ production, as described in the previous sections. It would seem logical that the neonate is developing an efficient system to increase intestinal absorption of calcium and phosphorus to maintain extracellular calcium levels without drawing on calcium and phosphorus from newly laid down bone.

Since the presence of calcium-binding protein (CaBP) is well correlated with calcium transport, it has been used as an index of calcium transport across the intestine. Quantitation of calcium-binding protein in neonates has been limited to the rat and chick. Moriuchi and DeLuca (1974) found that vitamin D-dependent CaBP was undetectable in chick embryonic intestine, but increased within hours

of hatching and reached maximal levels within 24 hours. Bruns et al. (1979) did not find a postnatal increase in CaBP in neonatal rats until the rats were weaned at 18 days of age. They did show that injection of 1,25-$(OH)_2D_3$ into the peritoneal cavity of the 15-day-old rat would stimulate the production of CaBP prior to the normal onset of CaBP production at weaning. Bruns et al. (1979) put forward the hypothesis that chicks try to absorb calcium from a relatively poor source of calcium; i.e., grain, and must therefore increase gut absorption of calcium tremendously in order to survive. However, in the case of the rat, the milk is a very good source of calcium, and the suckling rat probably gets enough calcium by passive diffusion alone without a need to increase intestinal calcium absorption efficiency. In fact, the lack of CaBP in the suckling rat may protect the rat from becoming hypercalcemic. Unfortunately, no data are available which show the 1,25-$(OH)_2D$ profile of the neonatal rat from birth to weaning. Perhaps the rat does not exhibit an increase in plasma 1,25-$(OH)_2D$ concentration until after it is weaned.

Suckling Effects

Milk is an excellent source of calcium and phosphorus. Milk from a cow contains approximately 0.12 gm of calcium, 0.1 gm phosphorus, and 0.01 gm magnesium per 100 gm. Of the total calcium of cow's milk, two-thirds is in the colloidal form as calcium caseinate, phosphate, and

citrate, and of the remaining one-third, 55% is bound by citric acid, 10% by phosphoric acid, and 35% exists in the ionic form. The calcium content of colostrum is somewhat higher, approximately 0.17 gm/100 gm. (Kon and Cowie, 1961). Human milk is much lower in both calcium and phosphorus compared to cow's milk, and there are reports in the literature of a phosphate depletion syndrome of breast-fed infants whose mother's milk is deficient in phosphorus (Sagy et al., 1980).

Milk also contains vitamin D activity. However, due to the quantities of other lipids in milk, vitamin D and its metabolites in milk are difficult to isolate and quantitate accurately. Sahashi et al. (1967) reported that vitamin D sulfate, a water-soluble form of vitamin D, was present at levels of 204 international units per liter of Holstein cow milk and 950 I.U. per liter of human milk. None of the milk analyzed was colostrum. Preliminary studies from the laboratory of Littledike and Horst (1980) have indicated that vitamin D and many of the known metabolites of vitamin D can be found in cow's milk, and that higher concentrations of these substances are found in colostrum.

Calcium and magnesium in milk are readily absorbed by suckling animals. The lactose in milk enhances the absorption of calcium (Kon and Cowie, 1961). Heeg (1980) studied the concentration of calcium in portal vein blood of 100-150 kg. calves and noted an increase in portal vein calcium within one-half hour of ingestion of milk. By two hours, the plasma calcium in the portal vein and aorta had reached maximal levels. Avioli (1972) concluded that calcium is transported in

the duodenum of an active carrier-mediated, energy-dependent process; in the more distal segments of the intestine, the mechanism seems to passive transport and/or facilitated diffusion.

Vitamin D (and presumably its metabolites) in the diet is primarily absorbed by the distal ileum (Kodicek, 1960). It has also been demonstrated that bile salts are essential for absorption of vitamin D to occur (Schacter et al., 1964). Since the primary excretory route for vitamin D appears to be the bile (Heymann, 1937; Ponchon and DeLuca, 1969), it appears that there is a certain amount of entero-hepatic circulation of vitamin D (Kumar et al., 1980). Haldimann et al. (1980) conducted a recent study of 25-OHD_3 absorption following a single oral dose of 25-OHD_3 in adult humans. They found that there was a peak in plasma 25-OHD_3 approximately 6 hours after the ingestion of 200 µg 25-OHD_3. However, the greatest increment in the value of 25-OHD_3 in the plasma occurred during the first 2 hours following ingestion of 25-OHD_3.

No studies have been done on vitamin D absorption from colostrum by the neonate. During the first 18-24 hours after birth in the calf, the small intestine epithelial cells lack tight junctions between them and the cells are highly pinocytotic, allowing absorption of proteins and perhaps other substances found in colostrum (Schultz, 1973). It seems likely that in the calf and some other young animals the absorption of calcium, phosphorus, and vitamin D is much faster than in the older animal.

As yet, there have been no studies which have tried to relate maternal, placental, and maternal milk contributions of vitamin D to calcium and phosphorus homeostasis and vitamin D metabolism of the neonate.

SECTION I. VITAMIN D STATUS OF THE COW AT PARTURITION AND ITS INFLUENCE ON THE VITAMIN D STATUS OF THE NEWBORN CALF

ABSTRACT

Concentrations of calcium, phosphorus, magnesium, hydroxyproline, vitamin D, and vitamin D metabolites were determined on plasma samples from cows and their calves at the time of birth. The cows sampled exhibited a diverse vitamin D status at the time of parturition. Neonatal plasma calcium (11.29 ± 1.1 mg%), phosphorus (6.38 ± 1.29 mg%), and hydroxyproline (9.24 ± 2.65 μg%) concentrations at birth were significantly higher than in the dams at parturition and were not correlated with maternal concentrations of these elements. Maternal and neonatal magnesium concentration did not differ statistically and showed no significant correlation. There was a high degree of correlation between cow and neonatal calf plasma 25-hydroxyvitamin D_2 (25-OHD_2) ($r = 0.733$), 25-hydroxyvitamin D_3 (25-OHD_3) ($r = 0.888$), 24,25-dihydroxyvitamin D_2 [24,25-$(OH)_2D_2$] ($r = 0.770$), 24,25-dihydroxyvitamin D_3[24,25-$(OH)_2D_3$] ($r = 0.629$), and 25,26-dihydroxyvitamin D_3 [25,26-$(OH)_2D_3$] ($r = 0.840$). No correlation could be demonstrated between dam and calf 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$], vitamin D_3, and total vitamin D. Vitamin D_2 and 25-hydroxyvitamin D_3-26,23 lactone were not detectable in calf plasma. The concentration of 1,25-$(OH)_2D$ measured in the calves at birth was consistently low (41.2 ± 14.7 pg/ml). The 1,25-$(OH)_2D$ concentrations found in the dams spanned a wide range of values (16.1-261.7 pg/ml). We conclude that the vitamin D status of the dam is important in determining neonatal calf plasma concentrations of 25-OHD, 24,25-$(OH)_2D$, and 25,26-$(OH)_2D$. However, the fetal and neonatal calf is capable of inde-

pendent control over its plasma calcium, phosphorus, and 1,25-$(OH)_2D$ concentrations as demonstrated by the lack of correlation between neonatal and dam plasma 1,25-$(OH)_2D$, calcium, and phosphorus concentrations.

Vitamin D has been shown to be metabolized by the liver to 25-hydroxyvitamin D (25-OHD) (Tanaka et al., 1973). The 25-OHD, under hypocalcemic or hopophosphatemic conditions, undergoes 1α-hydroxylation by the kidney to form 1,25-dihydroxyvitamin D ($1,25-(OH)_2D$)--the most biologically active form of vitamin D (Lawson et al., 1971). In normocalcemic or hypercalcemic states, 25-OHD can be hydroxylated at the 24 and 26 carbons to form 24,25-dihydroxyvitamin D ($24,25-(OH)_2D$) and 25,26-dihydroxyvitamin D ($25,26-(OH)_2D$). These two metabolites are considered by some (DeLuca, 1976) to be products of degradative pathways.

Most of the mono and dihydroxy forms of vitamin D have been shown to have antirachitic activity equal or greater than that of vitamin D itself (DeLuca, 1979). As a result of these findings, several of the vitamin D metabolites have been used as preventatives for parturient hypocalcemia in dairy cows (Olson et al., 1973; Hoffsis et al., 1978). In light of this recent research, we felt it important to determine how the plasma calcium, phosphorus, and vitamin D status of the dam at parturition affects the plasma concentration of calcium, phosphorus, and vitamin D metabolites in the neonatal calf.

Calcium and phosphorus concentrations in neonatal plasma are considerably higher than in the maternal plasma at parturition (Malan, 1928; Garel and Pic, 1972; Barlet et al., 1978). Plasma calcium concentration of the fetus is independent of maternal plasma calcium. Bawden and Wolkoff (1967) infused calcium into the circulation of pregnant ewes and detected no change in fetal plasma calcium. Barlet et al. (1977) have

shown that hypocalcemia in cows suffering from parturient paresis does not affect plasma calcium of the fetal calf. However, these studies involved only transient changes in blood calcium concentration of the dam. Early work on rickets and fetal mineralization showed that long-term calcium and phosphorus deficiency of the mother resulted in neonates with abnormally low blood calcium and phosphorus concentrations (Sontag et al., 1936; Maxwell, 1935).

[^{3}H]-vitamin D_3 and [^{3}H]-25-OHD_3 have been shown to cross the placenta of pregnant rats (Haddad et al., 1971) and ewes (Ross et al., 1979). Ross et al. (1979) also showed that [^{3}H]-1,25-$(OH)_2D_3$ could be recovered from fetal lamb plasma following administration of [^{3}H]-1,25-$(OH)_2D_3$ to the ewe. However, Noff and Edelstein (1978) found that very little [^{3}H]-1,25-$(OH)_2D_3$ could be found in rat fetuses after administration of [^{3}H]-1,25-$(OH)_2D_3$ to the pregnant rat. They also report that the major portion of the radioactivity found in the fetus was associated with an esterified product of [^{3}H]-1,25-$(OH)_2D_3$.

The present paper reports the plasma concentration of calcium, phosphorus, magnesium, hydroxyproline, vitamin D_2 and D_3, 25-OHD_2, 25-OHD_3, 24,25-dihydroxyvitamin D_2 [24,25-$(OH)_2D_2$], 24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2D_3$], 25,26-dihydroxyvitamin D_3 [25,26-$(OH)_2D_3$], and of a recently discovered metabolite 25-hydroxyvitamin D_3-26,23-lactone (lactone) in neonatal calves and their dams at parturition.

MATERIALS AND METHODS

Heparinized blood samples were taken from ten Jersey, six Holstein, one Brown Swiss, one Ayrshire, and one Angus cows and their colostrum-free calves immediately at parturition via jugular puncture. Nine of the Jersey cows had received up to 125 mg (5×10^6 IU) of vitamin D_3 approximately thirty days prior to parturition. Previous work (Horst and Littledike, 1979a) on the use of vitamin D in the prophylaxis of postparturient hypocalcemia had indicated that these doses would alter the vitamin D and vitamin D metabolite status of dams considerably at the time of parturition. Cows were from the National Animal Disease Center and Iowa State University dairy herds. The cows studied calved during all seasons of the year. Our hope was to sample dams with a diverse vitamin D status at the time of parturition. Plasma calcium and magnesium concentrations were determine by atomic absorption spectrophotometry (Perkin-Elmer Corp., 1965). Plasma phosphorus (Fiske and Subbarrow, 1925) and hydroxyproline (Bannister and Burns, 1970) were determined colorimetrically using an AutoAnalyzer.

The concentrations of vitamin D and its metabolites in the plasma were determined using the assay systems developed by Horst et al. (1979a,b 1980) and Eisman et al., (1976).

Briefly, the procedure involved extracting plasma lipid with diethyl ether and methanol:methylene chloride (1:3). The lipid extract was dried under nitrogen and subjected to Sephadex LH-20 chromatography using a solvent system of 1:1:9 chloroform:methanol:hexane. The column eluent was separated into three fractions based on polarity. Vitamin D_2 and D_3 elute

first and comprise fraction No. 1. Fraction No. 2 consists of 25-OHD_2 and 25-OHD_3. The third fraction contains the polar dihydroxylated metabolites of vitamin D_3 and lactone.

Fraction Analysis

Vitamin D_2 and D_3

The vitamin D fraction is further chromatographed on a Lipidex 5000 column equilibrated and developed with 5:95 chloroform:hexane. The vitamin D fraction from this column was then subjected to high pressure liquid chromatography (HPLC) on a Zorbax silicic acid column developed in 0.25:99.75 isopropanol:methylene chloride. Vitamin D_2 and vitamin D_3 comigrate on this column. Separation of vitamin D_2 from vitamin D_3 was achieved by reverse-phase HPLC on a Zorbax ODS column developed in 4:96 water:methanol. Both forms of vitamin D can then be quantitated by U.V. absorbance. Total vitamin D (D_2 + D_3) can be determined from the 0.25:99.75 isopropanol:methylene chloride Zorbax silicic acid column by a competitive protein binding assay using the vitamin D-binding protein of sheep plasma diluted 1:50,000.

25-OHD fraction

The 25-OHD_2 and 25-OHD_3-containing fraction was further chromatographed on a Sephadex LH-20 column developed in 50:50 chloroform:hexane. The 25-OHD fraction from this column was then subjected to HPLC on a Zorbax silicic acid column developed with 4:96 isopropanol:hexane. This separates 25-OHD_2 from 25-OHD_3, allowing quantitation by U.V. absorbance.

Dihydroxylated vitamin D metabolites and lactone fraction

The third fraction from the initial Sephadex LH-20 column was subjected to HPLC on a Zorbax silicic acid column developed in 11:89 isopropanol:hexane. The eluent was collected as three fractions, the first of which consisted of 24,25-$(OH)_2D_2$, 24,25-$(OH)_2D_3$, 25,26-$(OH)_2D_2$, and lactone. These metabolites were separated from one another by further HPLC on a Zorbax silicic acid column developed in 3.5:96.5 isopropanol: methylene chloride.

The second and third eluent fractions collected from the 11:89 isopropanol:hexane Zorbax silicic acid HPLC column were 25,26-$(OH)_2D_3$ and 1,25-$(OH)_2D$, respectively.

After their final purification, all the dihydroxylated metabolites, except 25,26-$(OH)_2D_2$, and lactone were measured by competitive protein binding assays (Eisman et al., 1976; Horst et al., 1979a).

RESULTS

Maternal and Neonatal Plasma Concentrations of Calcium, Phosphorus, Magnesium and Hydroxyproline

Mean neonatal calf and cow plasma calcium, phosphorus, magnesium and hydroxyproline concentrations are presented in Table 1. Wide ranges in the dam's plasma calcium ($\bar{x} \pm$ S.D.) (7.88 ± 1.16 mg %) and phosphorus (3.41 ± 1.09 mg %) due to varying degrees of parturient paresis were observed. Neonatal plasma calcium (11.29 ± 1.1 mg %) and phosphorus (6.38 ± 1.29 mg %) were significantly higher ($P < .01$) than in the dams and were not correlated with maternal levels of these elements. Maternal and neonatal magnesium concentration did not differ statistically and showed no significant correlation. Neonatal hydroxyproline levels (9.24 ± 2.65 μg %) were significant higher than those of the dams (1.86 ± .7 μg %) ($P < .01$).

Treatment of the dams with vitamin D_3 thirty days prepartum had no significant effects on calf plasma calcium, phosphorus, magnesium, or hydroxyproline levels, as shown in Table 2.

Maternal and Neonatal Plasma Concentrations of Vitamin D_2 and Vitamin D_3 and their Metabolites

Although we had hoped that vitamin D treatment of some of the cows prepartum would result in higher vitamin D and vitamin D metabolite levels in these cows, we found that the vitamin D treatment caused a significant increase in the concentration of circulating vitamin D only. However,

Table 1. Plasma concentrations of calcium, phosphorus, magnesium, and hydroxyproline in cows and neonatal calves (mean ± S.D.)

	Maternal concentration	Neonatal concentration	Correlation coefficient
Calcium (mg/100 ml)	7.88 ± 1.16 n = 19	11.29 ± 1.08 n = 19	.025 P ≥ .05
Phosphorus (mg/100 ml)	3.41 ± 1.09 n = 17	6.38 ± 1.29 n = 17	.248 P ≥ .05
Magnesium (mg/100 ml)	2.04 ± 0.42 n = 18	2.03 ± 0.32 n = 18	.307 P ≥ .05
Hydroxyproline (μg/100 ml)	1.86 ± 0.70 n = 16	9.24 ± 2.65 n = 16	.169 P ≥ .05

Table 2. Plasma concentrations of calcium, phosphorus, magnesium, and hydroxyproline in cows and the calves born to them grouped according to vitamin D_3 treatment of dam prepartum (mean ± S.D.)

Vitamin D dose	Ca^{++}		Phos		OH-Prol		Mg^{++}	
	Cow	Calf	Cow	Calf	Cow	Calf	Cow	Calf
				mg/100 ml				
0	8.42 ±.62	11.67 ±1.27	4.07 ±1.00	6.5 ±1.61	2.28 ±.70	8.66 ±2.39	1.86 ±.39	2.02 ±.31
	n=10	n=10	n=9	n=9	n=9	n=9	n=10	n=10
2.5×10^6 IU	6.8 ±.26	11.23 ±.86	2.67 ±.47	6.6 ±1.2	1.27 ±.23	9.47 ±4.27	2.07 ±.20	2.1 ±.49
	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3
2.5×10^6 IU x 2	6.2 ±1.15	11.03 ±.46	2.53 ±.91	6.47 ±.15	1.40 ±.36	11.2 ±1.9	2.64 ±.31	2.15 ±.37
	n=3	n=3	n=3	n=3	n=3	n=3	n=3	n=3
5×10^6 IU	8.75 ±.21	10.5 ±.14	2.8 ±.14	5.4 ±.85	1.3	7.9	1.9	1.86
	n=2	n=2	n=2	n=2	n=1	n=1	n=1	n=1

there were breed, herd, ration, susceptibility to milk fever, and seasonal differences among the cows resulting in wide ranges of concentrations of the vitamin D metabolites.

The concentration of vitamin D_2 and vitamin D_3 and their metabolites measured in the cows and calves at parturition are presented in Table 3. These data represent the first measurement of vitamin D_2 or vitamin D_3 in the neonatal calf and the first measurements of the vitamin D_2 metabolites of any neonate.

From the data presented, we observed a high degree of correlation between cow and neonatal calf plasma 25-OHD_2 ($r = 0.733$), 25-OHD_3 ($r = 0.888$), 24,25-$(OH)_2D_2$ ($r = .770$), 24,25-$(OH)_2D_3$ ($r = .629$), and 25,26-$(OH)_2D_3$ ($r = .840$). No correlation could be demonstrated between dam and calf 1,25-$(OH)_2D$, vitamin D_3, and total vitamin D. Vitamin D_2 and lactone were not detectable in calf plasma. Lactone was detected only in those cows that had received vitamin D_3 prepartum.

The concentration of 1,25-$(OH)_2D$ measured in the calves was consistently low (41.2 ± 14.7 pg/ml). The 1,25-$(OH)_2D$ concentrations found in the dams covered a wide range of values (16.1-261.7 pg/ml) as a result of clinical parturient hypocalcemia observed in several of the cows (Horst et al., 1977).

Table 3. Plasma concentrations of vitamin D_2 and D_3 and their metabolites in cows and the calves born to them and coefficient of correlation between maternal and neonatal plasma concentrations (mean S.D.)

Vitamin D metabolite	Maternal	Neonatal	Correlation coefficient
Vitamin D_2 (ng/ml)	0.28 ± 0.58 (9)	<0.1 (10)	<0.1 P > .05
Vitamin D_3 (ng/ml)	11.63 ± 12.76 (9)	0.13 ± 0.26 (9)	-0.161 P > .05
25-OHD_2 (ng/ml)	10.55 ± 9178 (19)	2.17 ± 1.39 (18)	0.733 P < .001
25-OHD_3 (ng/ml)	73.07 ± 41.74 (19)	22.23 ± 15.72 (18)	0.888 P < .0001
1,25-$(OH)_2D$ (pg/ml)	84.57 ± 68.65 (19)	42.61 ± 14.27 (18)	0.219 P > .05
24,25-$(OH)_2D_2$ (ng/ml)	0.41 ± 0.63 (15)	0.39 ± 0.57 (14)	0.770 P < .01
24,25-$(OH)_2D_3$ (ng/ml)	5.15 ± 5.99 (17)	4.36 ± 3.60 (16)	0.629 P < .05
25,26-$(OH)_2D_3$ (ng/ml)	2.93 ± 2.84 (17)	2.44 ± 1.61 (16)	0.840 P < .0001
Lactone	1.09 ± 2.26 (13)	<0.1 (13)	<0.1 P > .05

DISCUSSION

The developing fetus requires the translocation of large amounts of calcium and phosphorus from the maternal circulation across the placenta. A distinct fetal hypercalcemia and hyperphosphatemia relative to adult cow plasma calcium concentration was seen in the calves studied. Our observations corroborate those of earlier workers (Malan, 1928; Garel and Pic, 1972; Barlet et al., 1979) who showed that calcium and phosphorus are transported across the placenta against a concentration gradient. The poor correlation between maternal and neonatal plasma calcium, phosphorus, and 1,25-$(OH)_2D$ (the active form of vitamin D important in calcium homeostasis) suggests the fetus has independent control of calcium homeostasis at term.

The lack of a correlation between maternal and neonatal calf vitamin D_2 and D_3 concentrations in the plasma requires further explanation. Vitamin D is rapidly cleared from the blood by the liver where it is sequestered. As a result, plasma concentrations of vitamin D are usually low and in many cases undetectable. Therefore, the poor correlation between maternal and neonatal concentrations of vitamin D_2 and D_3 do not necessarily reflect the ability of vitamin D to cross the placenta. In addition, the feto-placental unit may be capable of sulfating or esterifying vitamin D, as has been shown in the case of estrogen (Levitz et al., 1961). Vitamin D concentrations alone are not generally considered a good indication of the vitamin D status of the animal (Horst and Littledike, 1979a). There was a significant degree of correlation between maternal and neonatal concentrations of 25-OHD_3, 24,25-$(OH)_2D_3$, and 25,26-$(OH)_2D_3$.

Barlet et al. (1978) and Ross et al. (1976), working with sheep, and Hillman and Haddad (1974), working with humans, have also demonstrated that the plasma concentration of 25-OHD_3 in neonatal plasma is highly dependent on 25-OHD_3 concentration of the maternal circulation. In contrast to our findings, Hillman et al. (1978) did not detect a significant maternal-neonatal correlation for 24,25-$(OH)_2D_3$. We also are reporting the findings that 25-OHD_2 and 24,25-$(OH)_2D_2$ concentrations in the neonate are correlated with maternal concentrations. These data represent the first measurement of any vitamin D_2 metabolites in the neonate as influenced by maternal plasma concentrations.

In all of the cow-calf pairs studied, 25-OHD_2 and 25-OHD_3 concentrations of the dam were higher than in the corresponding neonate. Coupled with the high degree of correlation between dam and calf 25-OHD concentrations, this suggests that 25-OHD_2 and 25-OHD_3 passively diffuse across the placenta.

Nine of the nineteen calves had 24,25-$(OH)_2D_3$ concentrations which were higher than their dam. Four of the calves had 24,25-$(OH)_2D_2$ concentrations higher than that of their dams, and six of the calves had 25,26-$(OH)_2D_3$ concentrations in excess of their dams. Placental transfer of these metabolites from the dam to the fetus may account for a portion of the high concentrations in the neonate. However, unless the placenta actively transports these metabolites against a concentration gradient, it must be assumed that much of the 24,25-$(OH)_2D_2$, 24,25-$(OH)_2D_3$, and 25,26-$(OH)_2D_3$ measured was of fetal origin.

The metabolism of vitamin D to the hormonally-active form [1,25-$(OH)_2D$] is presently recognized to be regulated by the concentration of calcium, phosphorus, and parathyroid hormone in the blood (DeLuca, 1979). Neonatal and maternal blood differ markedly in the concentration of these factors, primarily due to the active transport of calcium and phosphorus across the placenta. The result is a maternal compartment which is low in calcium and phosphorus (due to fetal and lactational drain of these elements) and normal or high in parathyroid hormone concentration (Hillman et al., 1978). In contrast, the fetus is well supplied with calcium and phosphorus, and cord serum shows a low level of parathyroid hormone present at birth (Hillman et al., 1978). As a result of these differences, maternal vitamin D metabolism tends toward production of 1,25-$(OH)_2D$ while in the fetus, 24,25-$(OH)_2D$ seems to be the major dihydroxylated form. Lester et al. (1978) have demonstrated that fetal rat plasma concentrations of 1,25-$(OH)_2D$ and 24,25-$(OH)_2D$ are maintained after maternal nephrectomy, suggesting that the feto-placental unit is exerting independent control of the metabolism of 25-OHD.

While it is widely believed that 24-hydroxylation and 26-hydroxylation are major paths in the deactivation and excretion of vitamin D metabolites, there is evidence that 24,25-$(OH)_2D$ is essential to normal mineralization of bone in man (Rasmussen and Bordier, 1978) and in upper mandibular development in embryonic chicks (Henry and Norman, 1978).

Despite the report by Ross et al. (1979) that 1,25-$(OH)_2D$ readily crosses the placenta, we could not find any correlation between maternal and neonatal 1,25-$(OH)_2D$ plasma concentrations. 1,25-$(OH)_2D$ concentra-

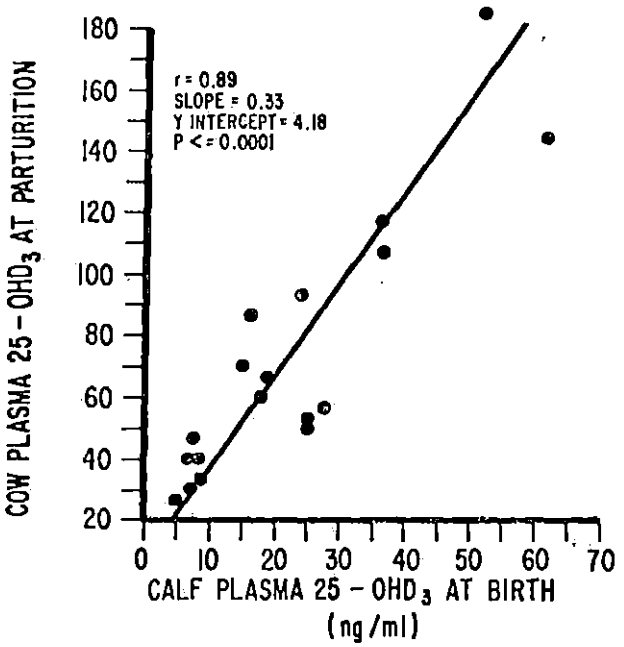

Figure 1. Cow plasma concentration of 25-OHD_3 at parturition versus calf plasma concentrations of 25-OHD_3 at birth.

trations of human infants are also independent of maternal concentrations of 1,25-$(OH)_2D$ (Steichen et al., 1980). These discrepancies may reflect differences in placentation between species. Noff and Edelstein (1978) report that 1,25-$(OH)_2D$ may be esterified by the rat fetus. This may represent one means of protection from high maternal concentrations of 1,25-$(OH)_2D$ if it can be inactivated after it crosses the placenta.

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SECTION II. MINERAL AND VITAMIN D METABOLISM OF THE NEONATAL CALF

ABSTRACT

Plasma samples were obtained from ten neonatal calves at 0, 24, 48, 77, and 98 hours following birth. Plasma calcium concentration was 11.6 ± 0.4 mg/100 ml at birth. During the first 24 hours following birth there was a significant decline in plasma calcium to 10.1 ± 0.3 mg/100 ml. By 48 hours of age plasma calcium had increased to near birth concentration (10.7 ± 0.3 mg/100 ml). Plasma phosphorus and magnesium concentrations during the sampling period did not change significantly from birth concentrations.

Plasma 1,25-$(OH)_2D$ concentration was very low at birth (48 ± 4 pg/ml) but rose significantly during the first 24 hours following birth (116 ± 19 pg/ml) and continued to rise to a maximum of 156 ± 39 pg/ml at 48 hours of age. By 77 hours 1,25-$(OH)_2D$ concentration declined to 103 ± 12 pg/ml.

Plasma 25-OHD, 24,25-$(OH)_2D$, and 25,26-$(OH)_2D$ concentrations did not change significantly from birth concentrations during the first 98 hours following birth. Plasma vitamin D was undetectable in all calves at birth but rose to significant concentrations in those calves fed colostrum from vitamin D treated dams during the sampling period.

We conclude that separation of the neonate from the placenta results in a decline in plasma calcium concentration which in turn results in an increase in 1-α hydroxylase activity and production of 1,25-$(OH)_2D$ to increase the plasma calcium concentration of the neonate.

INTRODUCTION

Once the umbilicus has been severed or the egg shell broken, the neonate can no longer rely on the placenta or magma of the egg to supply it with calcium and phosphorus. If the neonate is to adapt to extrauterine life, it must obtain calcium and phosphorus from its diet; i.e., meat and grain in the case of birds and milk in the case of mammals. The neonate must develop an efficient system of intestinal absorption of calcium and phosphorus if it is to maintain extracellular concentrations of these elements without having a net resorption of newly developed bone.

The vitamin D endocrine system has proved to play an important role in calcium homeostasis. Vitamin D, through one of its active metabolites 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$), initiates the active transport of calcium and phosphate across the intestinal epithelium (DeLuca, 1979) and is necessary for resorption of bone (Rasmussen and Bordier, 1978).

The purpose of this study was to characterize the changes in plasma calcium, phosphorus, magnesium, and some of the vitamin D metabolites of neonatal calves at birth and during the first few days of life - a critical period for the neonate in terms of calcium homeostasis.

MATERIALS AND METHODS

Heparinized blood samples (from the jugular vein) were taken from Holstein and Jersey calves at birth and at various intervals during the first few days following birth.

Several of the Jersey dams received doses of vitamin D_3 (up to 125 mg) intramuscularly approximately 30 days prior to calving. In a previous paper, we reported that at birth the calves from these dams did not differ statistically from calves from untreated dams with respect to plasma concentrations of calcium, phosphorus, magnesium, and vitamin D and its metabolites (Goff et al., submitted for publication).

Plasma calcium and magnesium concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer Corp., 1965). Plasma phosphorus (Fiske and Subbarrow, 1925) and hydroxyproline (Bannister and Burns, 1970) were determined colorimetrically.

Plasma concentrations of vitamin D_2, vitamin D_3, 25-hydroxyvitamin D_2 (25-OHD_2), 25-hydroxyvitamin D_3 (25-OHD_3), 24,25-dihydroxyvitamin D_2 [24,25-$(OH)_2D_2$], 24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2D_3$], 1,25-$(OH)_2D$, and 25,26-dihydroxyvitamin D_3 [25,26-$(OH)_2D_3$] were measured using the assay of Horst et al. (1980).

The calves were fed colostrum (5% of the calf birth weight) within one hour of birth and at approximately 12-hr intervals thereafter. Blood samples were taken immediately before the calf was fed. Calves were removed from the experiment if they developed health problems such as scours.

RESULTS

Plasma concentrations of each parameter measured were grouped for statistical purposes at 0, 24 ± 0.8, 48 ± 1.3, 77 ± 1.9, and 98 ± 4.2 hours following birth. Mean ± standard error of the mean were determined for each parameter. Statistical analysis involved use of the two-tailed Students _t_ test.

Plasma Calcium, Phosphorus, Magnesium, and Hydroxyproline

Mean plasma calcium, phosphorus, magnesium, and hydroxyproline concentration at 0, 24 ± 0.8, 48 ± 1.3, 77 ± 1.9, and 98 ± 4.2 hours after birth are presented in Table 1. Plasma calcium concentration was 11.6 ± 0.4 mg/100 ml at birth. Within 24 hr after birth, there was a significant ($P < .01$) decline in plasma calcium to 10.1 ± 0.3 mg %. By 48 hr after birth, plasma calcium concentration had increased to the plasma calcium concentrations seen at birth. Plasma calcium concentrations remained relatively stable thereafter.

Plasma phosphorus and hydroxyproline concentrations were 6.6 ± 0.4 mg/100 ml and 10.2 ± 1.0 μg/ml at birth. These parameters did not change significantly ($P > .05$) during the first 4 days after birth. However, there was a tendency for both phosphorus and hydroxyproline to decline during the first 24 hr after birth, followed by an increase in plasma phosphorus and hydroxyproline by 77 hr of age (Table 1).

Plasma magnesium concentration remained constant during the first 4 days following birth.

Table 1. Calf plasma concentrations of calcium, phosphorus, magnesium and hydroxyproline during the first 4 days after parturition (mean ± standard error of mean)

	Hours after parturition				
	0	24±0.8	48±1.3	77±1.9	98±4.2
Calcium (mg/100 ml)	11.6±0.4 n=10	10.1±0.3 n=8	10.7±0.3 n=7	11.0±0.2 n=6	11.3±0.6 n=6
Phosphorus (mg/100 ml)	6.6±0.4 n=9	5.9±0.4 n=10	6.8±0.3 n=9	6.3±0.3 n=8	6.3±0.4 n=6
Magnesium (mg/100 ml)	2.2±0.1 n=10	2.2±0.1 n=8	2.3±0.1 n=7	1.9±0.1 n=7	2.0±0.1 n=6
Hydroxyproline (μg/100 ml)	10.2±1.0 n=9	7.8±0.7 n=9	8.3±1.2 n=9	10.3±0.8 n=9	9.3±1.2 n=6

Vitamin D and Vitamin D Metabolites

Mean concentrations of vitamin D and its metabolites at 0, 24 ± 0.8, 48 ± 1.3, 77 ± 1.9, and 98 ± 4.25 hours after birth are shown in Table 2. Except for vitamin D_3, the plasma concentrations of the vitamin D_3 metabolites were not affected by vitamin D_3 treatment of the dams. Therefore, the vitamin D_3 metabolite data from all the calves were pooled and averaged for statistical analysis.

Vitamin D

Vitamin D_2 was not detected in the plasma of these calves at birth or during the first 4 days after birth. At birth and during the subsequent 98 hr, vitamin D_3 was undetectable at all time points in the plasma of the calves whose dams had not received intramuscular injections of vitamin D_3. The vitamin D_3 concentration of calves whose dams had received vitamin D_3 was 0.1 ± 0.03 ng/ml at birth. By 48 hr of age, these calves had significantly elevated plasma vitamin D_3 concentrations ($P < .05$) reaching 1.5 ± 0.6 ng/ml. The plasma vitamin D_3 concentration continued to increase thereafter and began to plateau at 2.4 ± 0.7 ng/ml by 77 hr following birth (Fig. 1).

25-OHD_2 and 25-OHD_3

Mean plasma 25-OHD_2 and 25-OHD_3 concentrations at birth were 2.4 ± 0.5 ng/ml and 18.8 ± 3.7 ng/ml, respectively. There was no significant change in mean plasma concentration of 25-OHD_2 or 25-OHD_3 during the first 4 days after birth in normal, nonscouring calves.

During the course of these experiments, 2 calves developed scours. Blood sampling was discontinued shortly following the onset of the scours.

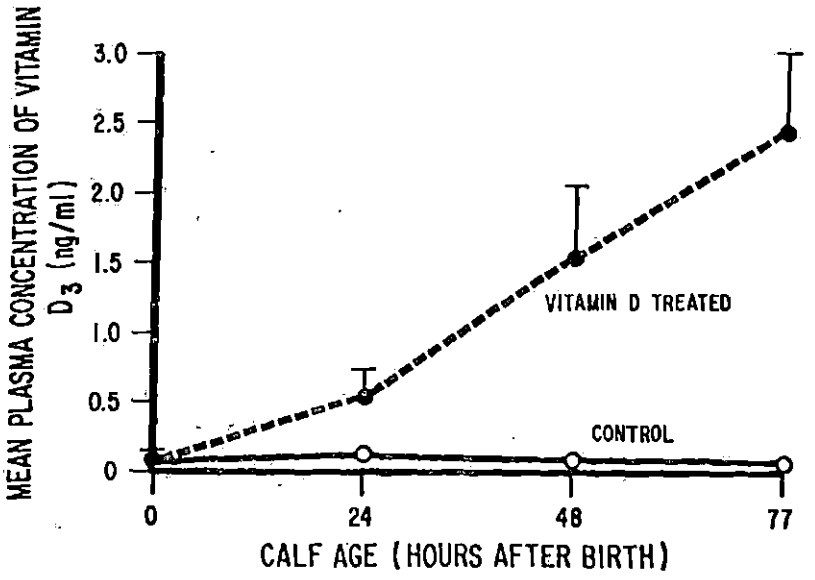

Figure 1. Plasma vitamin D_3 concentrations in calves fed colostrum from vitamin D treated (----) or control cows (——) during the 77 hours following birth. The standard error of the mean is indicated for each value where vitamin D was detectable. Hour 0 represents time of birth.

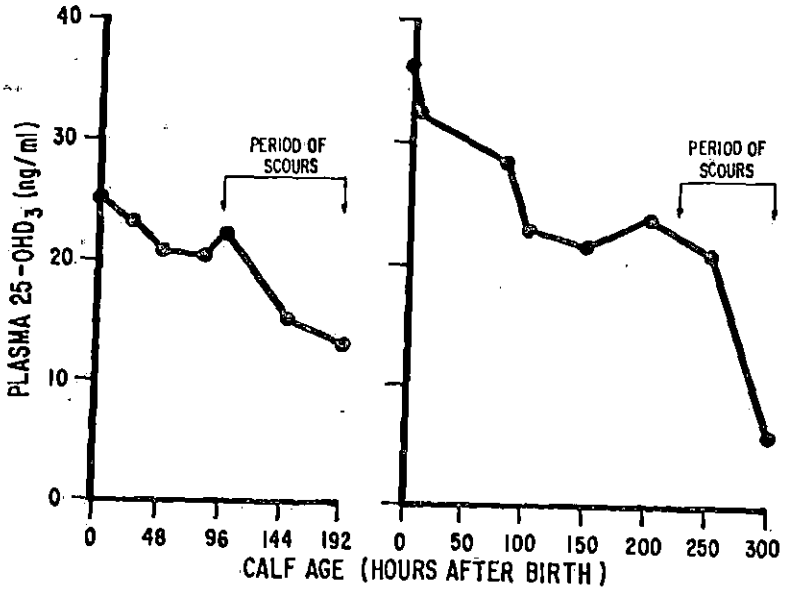

Figure 2. Plasma concentrations of 25-OHD_3 in two calves during the onset of scours. The period of scours is indicated by the arrows. Each calf had exhibited stable plasma 25-OHD_3 concentrations for at least 72 hours prior to the onset of scours.

Table 2. Calf plasma concentrations of vitamin D and vitamin D metabolites during the first 4 days after parturition (mean ± standard error of mean)

	Hours after parturition				
	0	24±0.8	48±1.3	77±1.9	98±4.2
Vitamin D_2 (ng/ml)	<0.1	<0.1	<0.1	<0.1	<0.1
Vitamin D_3 (ng/ml)	0.1±0.03 n=6	0.5±0.2 n=6	1.5±0.6 n=5	2.4±0.7 n=4	
25-OHD_2 (ng/ml)	2.4±0.5 n=10	2.2±0.6 n=10	1.9±0.6 n=9	2.1±0.6 n=9	2.6±0.7 n=6
25-OHD_3 (ng/ml)	18.8±3.7 n=10	18.8±4.6 n=10	17.1±4.2 n=9	19.0±3.6 n=8	21.0±3.8 n=6
1,25-$(OH)_2D$ (pg/ml)	48.0±4 n=10	116±19 n=10	156±39 n=9	103±12 n=8	105±20 n=6
24,25-$(OH)_2D_2$ (ng/ml)	<0.1	<0.1	<0.1	<0.1	<0.1
24,25-$(OH)_2D_3$ (ng/ml)	2.7±0.6 n=10	2.2±0.4 n=10	2.1±0.5 n=8	2.9±0.6 n=8	3.0±0.6 n=6
25,26-$(OH)_2D_3$ (ng/ml)	2.8±0.6 n=9	1.8±0.3 n=9	1.6±0.3 n=8	1.3±0.2 n=6	1.6±0.2 n=5

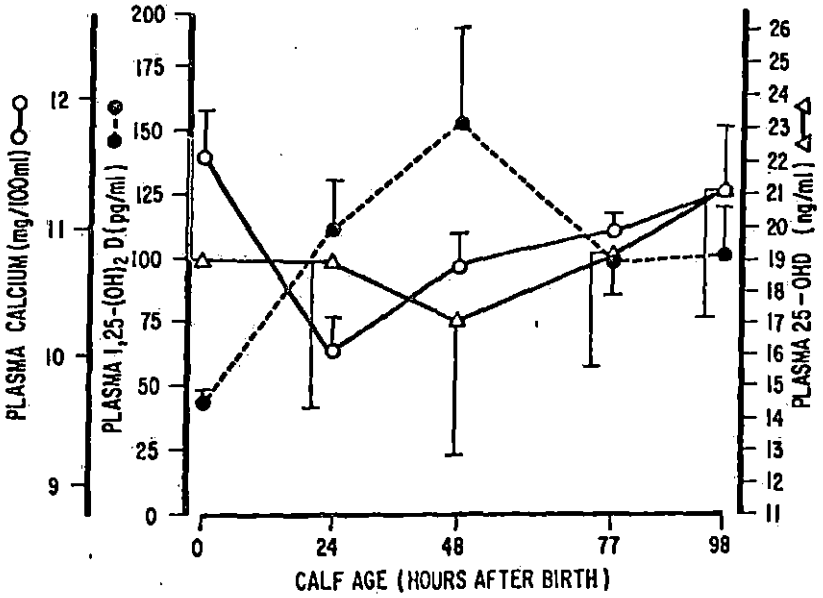

Figure 3. Mean plasma concentrations of calcium (o—o), 25-OHD (Δ—Δ), and 1,25-$(OH)_2$D (•--•) in neonatal calves. The standard error of the mean is indicated for each value.

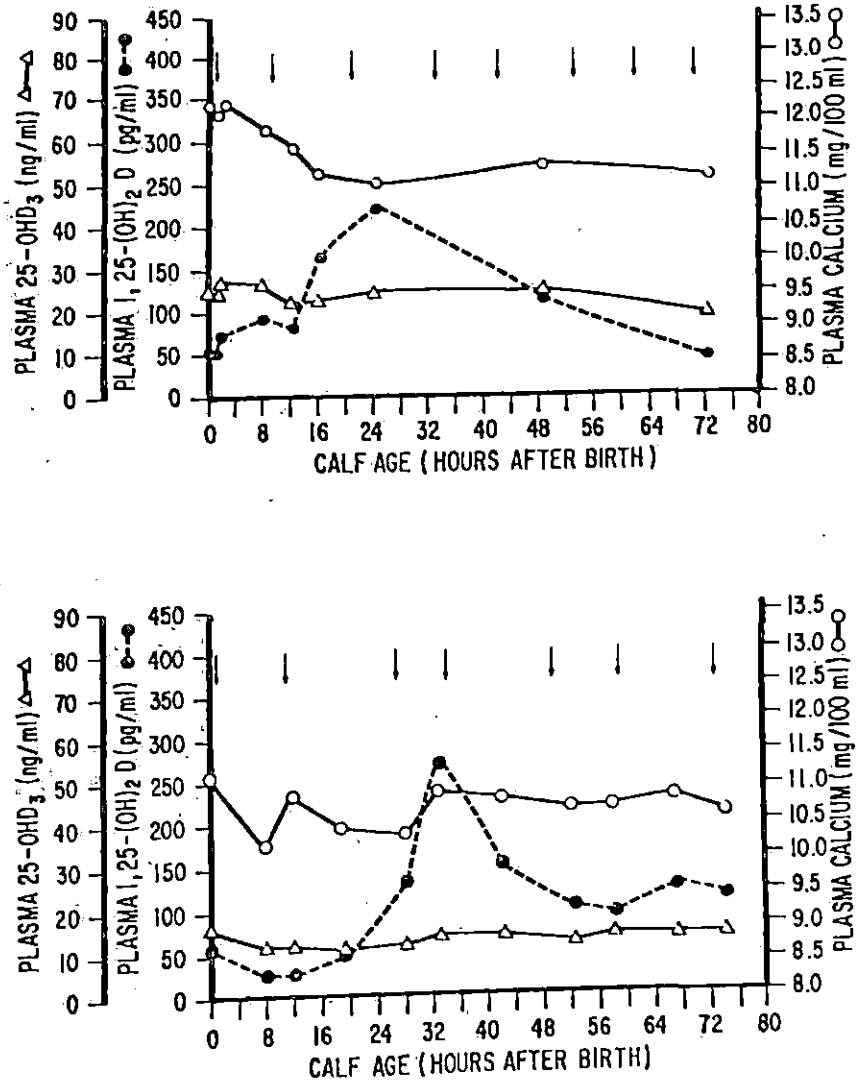

Figure 4. Individual calf plasma calcium (o—o), 25-OHD_3 (Δ—Δ), and 1,25-$(OH)_2D$ (•---•) concentrations during the neonatal period. Calves were fed at times indicated by arrows.

The results of 25-OHD_3 measurements made in plasma samples taken before and during the onset of the scours (Fig. 2) show plasma concentrations falling precipitously to approximately 50% of the prescours 25-OHD_3 concentrations.

Dihydroxylated metabolites of vitamin D

Plasma concentration of 1,25-$(OH)_2D$, the active form of vitamin D, was lowest at birth, averaging 48 ± 4 pg/ml. Within 24 hr after birth, plasma 1,25-$(OH)_2D$ concentration had significantly increased ($P < .001$) to 116 ± 19 pg/ml. Mean plasma 1,25-$(OH)_2D$ concentration continued to rise at 48 hr to 156 ± 39 pg/ml and remained significantly ($P < .01$) higher at the 77-hr sampling period (103 ± 12 pg/ml) (Table 2). The rising plasma concentrations of 1,25-$(OH)_2D$ were occurring during a period when plasma calcium was falling and plasma 25-OHD was constant (Figs. 3 and 4).

24,25-$(OH)_2D_2$ was detectable in only 2 of 10 calves at birth or during the first 3 days after birth and was present in these calves at very low concentration.

Plasma concentrations of 24,25-$(OH)_2D_3$ and 25,26-$(OH)_2D_3$ at birth were 2.7 ± 0.6 ng/ml and 2.8 ± 0.6 ng/ml, respectively. There was no significant change throughout the experiment in plasma concentration of either metabolite; however, both tended to decrease during the first 48 hr after birth.

DISCUSSION

Vitamin D metabolism is presently recognized to be regulated by the concentrations of calcium, phosphorus, and parathyroid hormone in the blood (Boyle et al., 1971; Garabedian et al., 1972; Rader et al., 1979). When compared with the dam, the fetus or neonate (0-2 hr of age) has higher plasma calcium and phosphorus concentrations (Barlet et al., 1979) due to active transport of these minerals by the placenta (Malan, 1928).

This high plasma calcium in the prenatal and neonatal (0-2 hr of age) calf probably results in correspondingly low plasma parathyroid hormone as has been demonstrated in newborn human infants (Hillman et al., 1978). The low plasma parathyroid hormone would result in lowered kidney 25-OHD-1α-hydroxylase activity and, therefore, could be responsible for the lower concentrations of 1,25-$(OH)_2$D detected in the newborn calf plasma in our experiment. However, by 24 hr of age, the plasma calcium concentration significantly fell below birth concentrations. As a result, parathyroid hormone production would be enhanced leading to a state of stimulated kidney 1α-hydroxylase activity and elevated plasma 1,25-$(OH)_2$D. The elevated plasma 1,25-$(OH)_2$D would result in increased intestinal calcium and phosphorus absorption and increased renal reabsorption of calcium and phosphorus (Steele et al., 1975). This series of biological responses to 1,25-$(OH)_2$D is probably responsible for the increased plasma calcium in the 48-hr-old calves. By 77 hr of age, most of the calves had stabilized their plasma calcium concentrations to very near the concentration seen at birth. As a result of the elevation in plasma

calcium, the plasma 1,25-$(OH)_2D$ concentration decreased (Fig. 3). Steichen et al. (1980) have reported a very similar situation in human infants.

Normal plasma calcium and phosphorus concentrations in the adult bovine are 10.20 and 5.16 mg/100 ml, respectively (Altman and Dittmer, 1961). In the adult bovine, 1,25-$(OH)_2D$ production is not significantly stimulated until plasma calcium and/or phosphorus are significantly below normal concentrations (Horst et al., 1977). The neonatal calf is hypercalcemic and hyperphosphatemic when compared to its dam. The lowest plasma calcium and phosphorus concentrations seen in the calf occur at 24 hr of age. The observed concentration (Table 1 and Fig. 3) are above normal plasma calcium and phosphorus concentrations of adults. The calf, however, still responds by increasing the production of 1,25-$(OH)_2D$ during the decline in plasma calcium that occurs during its first 24 hr of life. This indicates that the kidney 1α-hydroxylase is fully capable of responding to the decline in plasma calcium seen in the young calf (<72 hr old) even though it is stimulated under conditions (plasma calcium >10 mg/100 ml) in which 1α-hydroxylase activity would normally be inhibited in the mature animal.

The high plasma hydroxyproline concentration of the neonatal calves (compared to their dams) is indicative of an extremely high rate of growth and remodling of bone. Perhaps the calf must maintain elevated plasma calcium and phosphorus concentrations to ensure rapid bone growth.

The concentration of 25-OHD_2, 25-OHD_3, 24,25-$(OH)_2D_2$, 24,25-$(OH)_2D_3$, and 25,26-$(OH)_2D_3$ in the calf's plasma at birth are highly dependent on

the concentration of these metabolites in the dam's plasma at parturition (Goff et al., submitted for publication). Therefore, there was a great range in plasma concentration of these vitamin D metabolites at birth. As stated earlier, there was little influence of prepartum vitamin D_3 treatments of the dams on the plasma concentrations of these metabolites in the dams or their calves. Although there was a decline in the mean plasma concentration of 25-OHD_3, 24,25-$(OH)_2D_3$, and 25,26-$(OH)_2D_3$ during the first 3 days after parturition, it was not a significant decrease ($P > .05$). One parameter that was influenced by prepartum treatment of vitamin D_3 to the dams was the mean plasma vitamin D_3 concentration of the neonates. The plasma vitamin D_3 rose significantly during the first 77 hr after birth in calves whose dams had received vitamin D_3 (Fig. 1). The untreated dams and their calves had undetectable plasma vitamin D_3 at every sampling period (Fig. 1). The calves displaying the plasma vitamin D increase were housed indoors, which would eliminate ultraviolet irradiation as a source of vitamin D. Thus, we believe the increase in plasma vitamin D_3 may be attributed to absorption of vitamin D_3 from colostrum. This conclusion is supported by preliminary studies in our laboratory indicating that vitamin D and its metabolites can be detected in cow's milk and that colostrum from vitamin D-treated cows has vitamin D as its major vitamin D sterol. Vitamin D is found to be only a minor component of the vitamin D sterols found in colostrum from untreated cows (Horst et al., submitted for publication). The present study did not provide any control for a possible influence of vitamin D metabolites ingested with colostrum on the concentration of these metabolites in the calf plasma.

Two points, however, argue against any influence of vitamin D metabolites in colostrum on the plasma concentration of vitamin D metabolites in the neonatal calf. First, with the exception of vitamin D, the concentrations of vitamin D metabolites in colostrum ranged only between 1-10% of that found in the plasma of the mother (Horst et al., submitted for publication). Second, in several calves blood samples were taken frequently immediately before and after the calf was fed colostrum. In these calves no immediate change in the plasma concentrations of the vitamin D metabolites could be detected (Fig. 4). Changes in vitamin D_3 metabolites could be expected within 6 hr following ingestion of the metabolite (Haldimann et al., 1980). Therefore, we feel that the significant increase in plasma 1,25-$(OH)_2$D beginning 24 hr after birth is not due to 1,25-$(OH)_2$D that have been ingested with colostrum, but rather represents an increase in the activity of the 1α-hydroxylase enzyme.

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SUMMARY

The vitamin D status of the dam at parturition has a significant effect on the concentration of several important vitamin D metabolites in the newborn calf. There was a significant correlation between plasma 25-OHD_2, 25-OHD_3, 24,25-$(OH)_2D_2$, 24,25-$(OH)_2D_3$, and 25,26-$(OH)_2D_3$ concentrations in the dam at parturition and the calf at birth. There was no correlation between cow plasma 1,25-$(OH)_2D$, calcium, and phosphorus concentrations at parturition and those seen in the calf at birth.

The lack of correlation between cow and calf plasma 1,25-$(OH)_2D$, calcium, and phosphorus concentrations indicates that the fetus at term has independent control over its calcium homeostatic mechanisms. However, since 25-OHD is the precurser for 1,25-$(OH)_2D$, a major homeostatic hormone, it seems that the calf is dependent on the cow for the raw materials which it needs to control calcium homeostasis during the first few days of life.

Following birth the newborn calf experiences a decline in plasma calcium since it no longer receives calcium from the placenta. The decline in plasma calcium results in a marked increase in 1-α hydroxylase activity so that by the time the calf is twenty four hours old it has a significantly increased plasma 1,25-$(OH)_2D$ concentration. This in turn stimulates increased intestinal absorption of calcium resulting in a return of plasma calcium concentration to that exhibited at birth.

The data obtained also indicate that vitamin D is not transferred across the placenta. However, it does seem to enter the mammary gland of the cow and subsequently the colostrum. Therefore the calf is born with undetectable concentrations of vitamin D in its plasma. We found that those calves fed colostrum from cows exhibiting high plasma vitamin D

concentration exhibited an increased concentration of vitamin D in their plasma. This serves as evidence that colostrum can be an important source of vitamin D for the neonate.

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ACKNOWLEDGMENTS

I would like to thank the members of my committee, laboratory technicians and fellow graduate students for making my graduate education a truly enlightening experience.

I would especially like to thank Drs. Travis Littledike and Ron Horst: Travis, for the moments of inspiration and the new approaches to every problem I encountered; and Ron, for the ability to carry out those ideas. I also thank them for their friendship and for giving me the feeling that I was a fellow researcher rather than just a graduate student.

Many thanks to Joel Sommerfeldt, Cindy Hauber, Becky Lyon, Yung Ju Oh, Tim Reinhardt, and Annette Bates for their aid and moral support.

I also must mention the fantastic assistance I received from the ISU dairy herd personnel, especially Larry Madren. Also the help of Lynn Davis in caring for the neonatal calves.

Last but not least I want to thank Belinda for making my hours away from the lab as enjoyable as those I spent in the lab. Actually, a lot more.