Proc. Natl. Acad. Sci. USA
Vol. 76, No. 11, pp. 5549–5553, November 1979
Biochemistry

Vitamin D metabolism during pregnancy and lactation in the rat

(reproduction/calcium/phosphate)

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Contributed by H. F. DeLuca, August 8, 1979

ABSTRACT **The plasma concentration of the major vitamin D metabolites; 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and 24,25-dihydroxyvitamin D were measured during pregnancy and lactation in the adult female rat. The concentrations of these metabolites were also measured in rat pups during lactation and after weaning. The plasma concentration of 1,25-dihydroxyvitamin D in the adult female increases from a control value of 26 pg/ml to 86 pg/ml during the latter stages of pregnancy, reaches a peak of 158 pg/ml during lactation, and then returns to control levels by 3 weeks postweaning. Plasma concentrations of 24,25-dihydroxyvitamin D fall dramatically during pregnancy from a control level of 3.9 ng/ml to 1.6 ng/ml, remain low during lactation, and return to control levels by 3 weeks postweaning. In the neonatal rat pup at 14 days postpartum, 1,25-dihydroxyvitamin D plasma concentration is 25 pg/ml and 24,25-dihydroxyvitamin D concentration is 2.8 ng/ml. By day 25 postpartum, 1,25-dihydroxyvitamin D concentrations reached levels of 101 pg/ml, whereas 24,25-dihydroxyvitamin D concentrations fell to 1.9 ng/ml.**

The metabolism of vitamin D and its involvement in normal calcium/phosphate metabolism are well documented (1–5). Dietary changes in calcium and phosphate are compensated for by changes in the vitamin D endocrine system in such a way as to maintain a normal calcium and phosphate balance.

Pregnancy and lactation bring about dramatic changes in the calcium/phosphate homeostatic mechanism (6). Intestinal calcium transport (7) and bone resorption (8) increase to meet fetal and neonatal calcium and phosphate requirements. The subsequent changes in vitamin D metabolism, however, have not been exhaustively described. Recent reports indicate that the most active hormonal form of vitamin D, 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$], is increased during pregnancy and lactation (9, 10). There is also strong evidence that there are fundamental differences between maternal and fetal metabolism of vitamin D (11). These findings underscore the importance of establishing the plasma concentrations of the major vitamin D metabolites and their relation to each other during pregnancy and lactation. In this paper we report the plasma concentrations of 25-hydroxyvitamin D (25-OH-D), 1,25-$(OH)_2D$, and 24,25-dihydroxyvitamin D [24,25-$(OH)_2D$] during pregnancy, lactation, and the postweaning period in the adult rat as well as the levels of these metabolites in the neonatal rat pup.

MATERIALS AND METHODS

Female Holtzman rats (Holtzman Rat Co., Madison, WI) were obtained as weanlings and maintained on a diet containing 0.44% calcium and 0.30% phosphorus (12). In addition, animals were given 25 international units of vitamin D per day in 0.1 ml of cottonseed/soybean oil (Wesson). At 130 days of age, the females were mated with normal male breeders from Holtzman. The presence of sperm in vaginal smears was used to establish the first day of pregnancy.

At days 18 and 20 of pregnancy, at day 14 of lactation, at the time of weaning which was set at 25 days postpartum, and at 3 weeks postweaning, females were anesthetized with ether, an abdominal incision was made, and blood was obtained by puncture of the dorsal aorta. Pups from these females were killed at day 14 of lactation, at weaning, and at 3 weeks postweaning and blood was obtained as above. As a control, blood was also taken from a group of nonmated, age-matched females at the beginning and at the end of the experiment. No difference was seen in the plasma concentrations of calcium, phosphate, 25-OH-D, 1,25-$(OH)_2D$, or 24,25-$(OH)_2D$ between these groups and therefore all values were combined to form one control group.

Plasma samples were immediately analyzed for calcium and phosphate. Plasma calcium concentration was determined by diluting 0.1 ml of plasma with 1.9 ml of 0.1% aqueous $LaCl_3$ and by measuring the calcium concentration by atomic absorption spectroscopy. Phosphate concentrations in the plasma were measured by the method of LeBel (12). The remaining plasma from each female and pooled plasma samples from 2 to 5 pups were frozen and stored at −20°C. They were later analyzed for 25-OH-D, 1,25-$(OH)_2D$, and 24,25-$(OH)_2D$ by using established methods (13–15).

RESULTS

Vitamin D Metabolite Levels in Adults. In order to relate changes in vitamin D metabolite levels to changes in calcium and phosphate homeostasis during pregnancy and lactation, we measured concentrations of calcium and phosphate in plasma (Table 1). There is a slight but significant drop in plasma calcium concentration during the latter stages of pregnancy and during lactation. At the time of weaning and 3 weeks postweaning, plasma calcium levels returned to normal. Plasma phosphate levels on the other hand remained normal throughout pregnancy and lactation, were slightly elevated at weaning, and were normal by 3 weeks after weaning.

Fig. 1 illustrates the changes in plasma concentration of 25-OH-D seen throughout pregnancy and lactation. 25-OH-D levels were significantly reduced below control levels by the 20th day of pregnancy. They remained depressed during lactation and weaning, and were still significantly below control values 3 weeks after weaning.

The plasma concentrations of 1,25-$(OH)_2D$ and 24,25-$(OH)_2D$ are shown in Fig. 2. On day 18 of pregnancy, plasma concentrations of 1,25-$(OH)_2D$ were slightly elevated but not significantly different from control concentrations. Two days later, on the 20th day of pregnancy, 1,25-$(OH)_2D$ levels were definitely elevated and by day 14 of lactation 1,25-$(OH)_2D$

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Abbreviations: 25-OH-D, 25-hydroxyvitamin D; 1,25-$(OH)_2D$, 1,25-dihydroxyvitamin D; 24,25-$(OH)_2D$, 24,25-dihydroxyvitamin D.

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Table 1. Plasma concentrations of calcium and phosphate in adult female rats

Group	Number of animals	Calcium, mg/100 ml	Phosphate, mg/100 ml
Control*	10	10.7 ± 0.1	5.8 ± 0.4
Day 18 of pregnancy	3	10.5 ± 0.4	5.7 ± 0.5
Day 20 of prenancy	5	9.7 ± 0.2†	5.8 ± 0.6
Day 14 of lactation	5	9.8 ± 0.1†	6.1 ± 0.1
Weaning	5	10.3 ± 0.2	7.3 ± 0.6‡
3 weeks postweaning	5	10.8 ± 0.2	6.3 ± 0.5

Values are expressed as mean ± SEM.
* Control animals refer to nonmated, age-matched females.
† Significantly different from control values ($P < 0.001$) using Student's t test.
‡ Significantly different from control values ($P < 0.025$) using Student's t test.

concentrations had increased nearly 6-fold to a value of 158 pg/ml. During this same time period plasma concentration of 24,25-$(OH)_2D$ dropped dramatically from a control value of 3.9 ng/ml to 1.6 ng/ml at day 14 of lactation.

By weaning, or the 25th day postpartum, 1,25-$(OH)_2D$ levels began to fall and 24,25-$(OH)_2D$ concentrations began to rise. By 3 weeks postweaning, both 1,25-$(OH)_2D$ and 24,25-$(OH)_2D$ levels had returned to normal.

Vitamin D Metabolite Levels in Pups. The plasma concentrations of calcium and phosphate from pups at day 14 of lactation, at weaning, and at 3 weeks postweaning are shown in Table 2. By using plasma calcium and phosphate concentrations at day 14 of lactation as a base, we observed no change in plasma calcium levels at weaning, but a slight, significant drop in plasma phosphate from 10.0 mg/100 ml to 9.1 mg/100 ml was detected. By 3 weeks postweaning plasma calcium values reached 11.7 mg/100 ml and plasma phosphate concentrations rose to a level not significantly different from that seen at day 14 of lactation.

Table 2. Plasma concentrations of calcium and phosphate in neonatal and postweaned rat pups

Group	Number of animals	Calcium, mg/100 ml	Phosphate, mg/100 ml
Day 14 of lactation	15	11.0 ± 0.1	10.0 ± 0.1
Weaning	26	11.1 ± 0.1	9.1 ± 0.1*
3 weeks postweaning	14	11.7 ± 0.1†	9.6 ± 0.2

Values are expressed as mean ± SEM.
* Significantly different from day 14 of lactation ($P < 0.005$) using Student's t test.
† Significantly different from day 14 of lactation ($P < 0.001$) using Student's t test.

The plasma concentrations of 25-OH-D, 1,25-$(OH)_2D$, and 24,25-$(OH)_2D$ in neonatal and postweaned rat pups are given in Table 3. No significant change in the concentration of 25-OH-D was seen. 25-OH-D levels at all times were similar to those seen in adult females at weaning.

1,25-$(OH)_2D$ levels in the plasma, on the other hand, increased roughly 4-fold from 25 pg/ml at day 14 of lactation to 101 pg/ml at weaning. 24,25-$(OH)_2D$ levels during this same time fell significantly from 2.8 pg/ml to 1.9 pg/ml. By 3 weeks postweaning, 1,25-$(OH)_2D$ concentrations in the plasma were still elevated and 24,25-$(OH)_2D$ concentrations were still depressed.

DISCUSSION

Vitamin D Metabolism in the Adult. The slight but significant decrease in plasma calcium concentration seen in the later stages of pregnancy is similar to the changes seen in human pregnancy (16) but is not entirely consistent with the recent work of Pike *et al.* (10). Pike *et al.* report that, on day 21 of pregnancy in the rat, plasma calcium levels are the same as nonpregnant, age-matched controls. The reduced plasma concentration of calcium seen during lactation is, however, similar to that seen in previous reports (7, 9, 10). The reason for

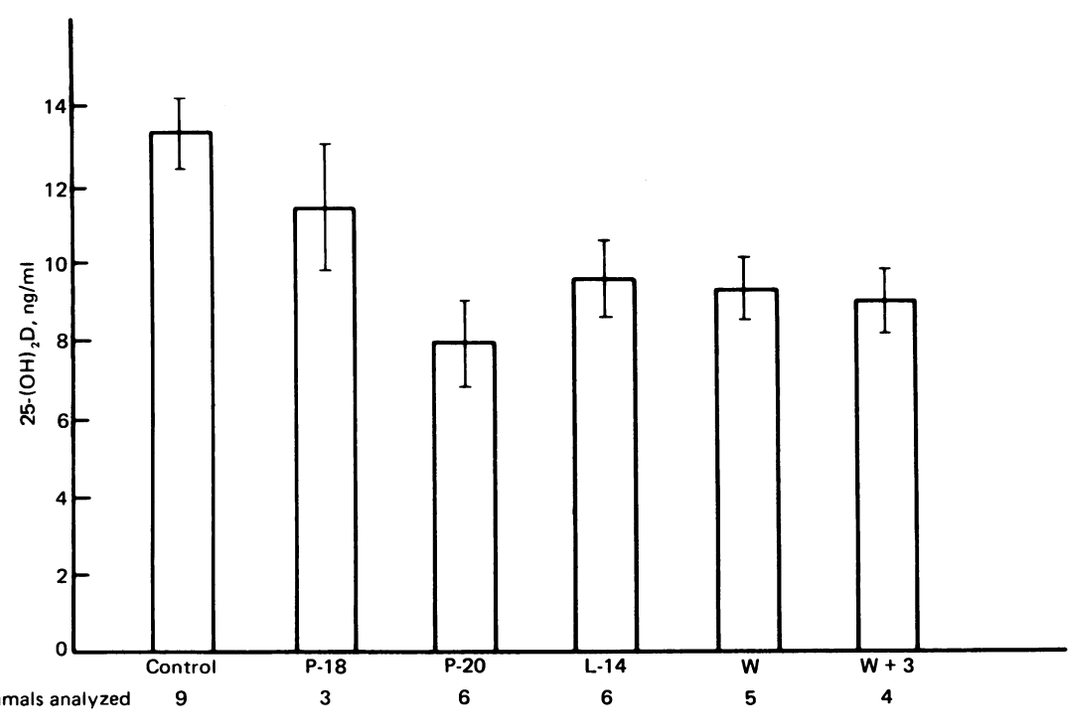

FIG. 1. Concentration of 25-OH-D in plasma from adult female rats. Plasma from nonmated, age-matched females (control) and plasma from females at day 18 (P-18) and day 20 (P-20) of pregnancy, at day 14 postpartum (L-14), at the time of weaning (W), and at 3 weeks postweaning (W + 3) were analyzed with established methods (13). Values are given as mean ± SEM. The concentration of 25-OH-D was significantly decreased to below the control level at P-20 ($P < 0.01$), L-14 ($P < 0.05$), W ($P < 0.025$), and W + 3 ($P < 0.025$) according to the Student's t test.

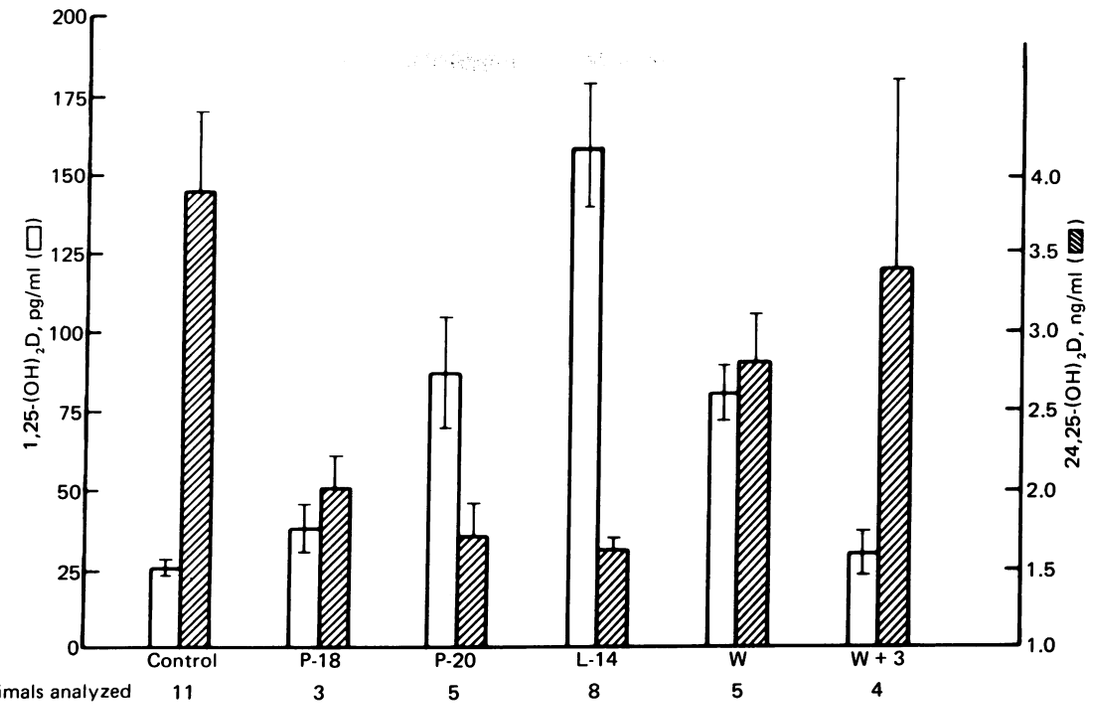

FIG. 2. Concentrations of 1,25-$(OH)_2D$ and 24,25-$(OH)_2D$ in plasma from adult female rats. Plasma samples from nonmated, age-matched females (control) and plasma from females at day 18 (P-18) and day 20 (P-20) of pregnancy, at day 14 postpartum (L-14), at the time of weaning (W), and at 3 weeks postweaning (W + 3) were analyzed with established methods (14, 15). Values are given as the mean ± SEM. According to the Student's *t* test, the concentration of 1,25-$(OH)_2D$ was significantly increased above the control level at P-20 ($P < 0.001$), L-14 ($P < 0.001$), and W ($P < 0.001$), whereas the concentration of 24,25-$(OH)_2D$ was significantly decreased to below the control level at P-18 ($P < 0.05$), P-20 ($P < 0.005$), and L-14 ($P < 0.005$). All other values were not significantly different from the control concentrations.

the decline in plasma calcium concentration during the later stages of pregnancy is most likely the increased flux of calcium into the fetus. Similarly, the decline seen in lactation is most likely the result of the efflux of calcium out of the plasma and into milk. The meaning of the plasma concentrations of calcium and phosphate at weaning requires some clarification. If rat pups are left with their mother indefinitely, the female will nurse them to approximately 28 days postpartum. From about day 16 postpartum to day 28 there is a gradual change in the dietary habits of the pup. At about day 16 or 17 the pup begins to consume some solid food but relies for the most part on maternal milk for nourishment. As the pup gets older his caloric consumption gradually shifts more and more toward solid food and away from milk. During this time, milk production and the subsequent calcium load on the plasma calcium pool also gradually decline. It is therefore likely that plasma concentrations of calcium and phosphate at weaning are critically dependent on the number of days postpartum chosen as the weaning date. For example, plasma calcium concentration in the mother has been reported to be decreased at day 20 postpartum (9) and from previous experiments in our laboratory at day 23 postpartum (17). In the present set of experiments pups were weaned at day 25 postpartum. As indicated in Table 1, plasma calcium in the mother has by this time returned to a normal level. The reason for the slight rise in plasma phosphate at the time of weaning is not clear but is consistent with previous reports (10).

Table 3. Plasma concentrations of 25-OH-D, 1,25-$(OH)_2D$, and 24,25-$(OH)_2D$ in neonatal and postweaned rat pups

Group	Number of samples*	25-OH-D, ng/ml	1,25-$(OH)_2D$, pg/ml	24,25-$(OH)_2D$, ng/ml
Day 14 of lactation	6	10 ± 1	25 ± 4	2.8 ± 0.3
Weaning	7	8 ± 1	101 ± 14†	1.9 ± 0.1‡
3 weeks post-weaning	7	9 ± 1	120 ± 19†	1.8 ± 0.3‡

* Plasma was pooled from two to five animals for each sample and values are expressed as mean ± SEM.
† Significantly different from day 14 of lactation ($P < 0.001$) using Student's *t* test.
‡ Significantly different from day 14 of lactation ($P < 0.05$) using Student's *t* test.

The changes seen in the plasma concentrations of 25-OH-D, 1,25-$(OH)_2D$, and 24,25-$(OH)_2D$ are complicated, and their interpretation is critical to an understanding of calcium and phosphate metabolism during pregnancy and lactation. It has been clearly shown that, under normal, nonpregnant, nonlactating conditions, 1,25-$(OH)_2D$ stimulates active calcium transport in the intestine (18, 19) and mediates bone mineral mobilization (20–22). The increase in plasma 1,25-$(OH)_2D$ levels during the later stages of pregnancy may in turn be the cause of the reported increase in intestinal calcium transport (7) and bone calcium mobilization seen in pregnancy (8). It is known, for example, that the greatest calcium influx into the fetus takes place late in pregnancy (6). The subsequent drain on the plasma calcium pool results in a drop in plasma calcium concentration (Table 1). This drop may in turn stimulate the renal 25-OH-D-1-hydroxylase via parathyroid hormone secretion. The increased hydroxylase activity could then account for the high plasma concentration of 1,25-$(OH)_2D$. In addition the placenta may contribute to circulating levels of 1,25-$(OH)_2D$ during pregnancy. It has been reported (23, 24) that anephric pregnant females still have the capacity to produce 1,25-$(OH)_2D$ through hydroxylation of 25-OH-D. Recent work in our laboratory has in turn shown definitively that the placenta can convert 25-OH-D into 1,25-$(OH)_2D$ (25). These re-

sults strongly support the hypothesis that the placenta plays a key role in vitamin D metabolism during pregnancy.

As is the case during late pregnancy, lactation puts a tremendous load on the plasma calcium pool. The efflux of calcium from the plasma into the milk decreases the plasma calcium concentration (Table 1) and this decrease likely accounts for the dramatic rise in plasma 1,25-$(OH)_2$D levels. The effect of the high circulating 1,25-$(OH)_2$D levels would be to stimulate intestinal transport of calcium and bone calcium mobilization to meet the calcium challenge of lactation.

As milk production begins to fall off later in lactation one would expect to see, and does in fact see, a return of plasma calcium concentrations to normal and a concomitant decrease in 1,25-$(OH)_2$D concentrations.

The function, if any, of 24,25-$(OH)_2$D during pregnancy and lactation is not clear. Generally speaking hydroxylation at the 24 position of vitamin D has been shown to reduce the biological activity of the molecule and may represent the initial step in a degradative pathway (26, 27). On the other hand it has been suggested that 24,25-$(OH)_2$D is important in bone mineralization (28), suppression of parathyroid hormone secretion (29), and embryonic development (30, 31). However, recent work with 25-OH-D chemically blocked at the 24 position with two fluorine atoms (24,24-difluoro-25-hydroxyvitamin D) does not support the idea that 24,25-$(OH)_2$D plays a major role in bone metabolism (32).

Under normal, nonpregnant, nonlactating conditions, the renal 1-hydroxylase and 24-hydroxylase and, hence, the plasma concentrations of 1,25-$(OH)_2$D and 24,25-$(OH)_2$D are strongly influenced by the plasma calcium concentration (33, 34). As the concentration of calcium in the plasma falls below ≈9.5 mg/100 ml, plasma levels of 1,25-$(OH)_2$D increase dramatically and levels of 24,25-$(OH)_2$D decrease, whereas, at plasma calcium concentrations greater than 9.5 mg/100 ml, 1,25-$(OH)_2$D concentrations in the plasma are low and 24,25-$(OH)_2$D concentrations are high. The changes in plasma concentrations of 24,25-$(OH)_2$D seen during pregnancy and lactation are consistent with these observations. As plasma calcium demands increase during late pregnancy and during lactation, 24,25-$(OH)_2$D levels fall and 1,25-$(OH)_2$D concentrations increase. Late in lactation and after weaning when calcium demands are diminishing, the opposite occurs.

It is interesting to note that the changes in plasma levels of 1,25-$(OH)_2$D and 24,25-$(OH)_2$D are not absolutely linked. In following the plasma changes in these metabolites through a reproductive cycle, we find that there is initially a drop in 24,25-$(OH)_2$D concentration with little or no change in the 1,25-$(OH)_2$D concentration. The 1,25-$(OH)_2$D level then gradually increases while the 24,25-$(OH)_2$D level remains low. After the peak in 1,25-$(OH)_2$D concentration during lactation there is a gradual decline in 1,25-$(OH)_2$D accompanied by a gradual, nearly linear increase in the 24,25-$(OH)_2$D concentration. This results in an asymmetry in the changes of these metabolites. The significance of this observation is not clear but may reflect an inherent property of the renal hydroxylase enzymes or the rates of degradation of 1,25-$(OH)_2$D and 24,25-$(OH)_2$D.

The function and control of 25-OH-D levels is poorly understood. The observation that the plasma concentration of 25-OH-D decreases late in pregnancy and remains at a lower level throughout lactation and even into the postweaning period may simply reflect the increased metabolic conversion of 25-OH-D to 1,25-$(OH)_2$D during this time.

Vitamin D Metabolism in the Rat Pup. Calcium and phosphorus requirements for growth and skeletal development are enormous in the growing rat and it would be expected that the vitamin D endocrine system would be shifted in such a direction as to maximally stimulate calcium transport in the intestine and bone formation. The high 1,25-$(OH)_2$D and the relatively low 24,25-$(OH)_2$D plasma levels at weaning and 3 weeks after weaning are consistent with this hypothesis. The relatively low concentration of 1,25-$(OH)_2$D during lactation appears strange but may be due to the effect of lactose on intestinal transport of calcium. Lactose has been shown to enhance the passive transport of calcium across the intestine (35). It may be that this effect coupled with the high concentrations of calcium and phosphorus in milk is sufficient to maintain a normal to slightly hypercalcemic (11.0 mg/100 ml) condition in the plasma and hence inhibit the activity of the renal 1-hydroxylase and stimulate the 24-hydroxylase. The net effect would be to decrease circulating levels of 1,25-$(OH)_2$D and increase levels of 24,25-$(OH)_2$D, exactly what is observed during lactation. As the animals' diet changes in the later stages of lactation and the consumption of milk, and therefore lactose, decreases, there should result a decrease in the passive flux of calcium out of the intestine and into the blood. This decrease may bring about a transitory fall in plasma calcium concentration and trigger an increase in renal 1-hydroxylase activity. This in turn could account for the high plasma levels of 1,25-$(OH)_2$D at weaning and 3 weeks postweaning.

The functions of 1,25-$(OH)_2$D during lactation and postweaning are not fully known. It is likely that 1,25-$(OH)_2$D stimulates intestinal calcium transport, at least in the later stages of lactation and after weaning. It is not clear, however, that the relatively low levels of 1,25-$(OH)_2$D measured in the pups during lactation are effective in stimulating transport. The low levels of circulating 1,25-$(OH)_2$D during lactation suggest that high plasma concentrations of 1,25-$(OH)_2$D are not necessary for proper bone growth and mineralization during this time.

The function and control of 24,25-$(OH)_2$D levels in the growing rat pup also remain obscure. The relationship between plasma concentrations of 1,25-$(OH)_2$D and 24,25-$(OH)_2$D is similar to that seen in older animals (33, 34) but with one fundamental difference. The plasma calcium threshold level for stimulation of 1,25-$(OH)_2$D production in the older animal is roughly 9.5 mg/100 ml whereas in the pup it is obviously greater than 11.7 mg/100 ml (Table 2). In other words, even in the face of hypercalcemic conditions, 1,25-$(OH)_2$D is preferentially being made over 24,25-$(OH)_2$D.

Undoubtedly 25-OH-D, 1,25-$(OH)_2$D, and 24,25-$(OH)_2$D play key roles in maintaining a normal pregnancy and lactation. Clearly, 1,25-$(OH)_2$D and 24,25-$(OH)_2$D levels undergo dramatic changes during the reproductive cycle. The reasons for these changes are not fully known. It is interesting to note however that even in the vitamin D-deficient state, under conditions in which 25-OH-D and 1,25-$(OH)_2$D are undetectable, that reproduction can still take place (17).

This work was supported by National Institutes of Health Program-Project Grant AM-14881 and Postdoctoral Fellowship AM05714 (B.H.), National Aeronautics and Space Administration Contract NAS-9-15580, and the Harry Steenbock Research Fund of the Wisconsin Alumni Research Foundation.

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