ages. The complete lack of altitude information severely limits our ability to make definitive statements about most of the features in these images. This problem will be at least partly solved by the Pioneer Venus orbiter, which is currently supplying altitudes at approximately 120-km spacings over the region covered by the Arecibo images. The combination of the two data sets should lead to a much better understanding of the nature of the large surface structures visible in the radar images.

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22 March 1979; revised 29 May 1979

Dynamic Changes in Circulating 1,25-Dihydroxyvitamin D During Reproduction in Rats

Abstract. *The concentrations of 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], calcium, and phosphorus were measured in the serum of rats during pregnancy and at various stages of lactation. The concentration of 1,25-$(OH)_2$D hormone increased almost twofold during pregnancy and the latter part of lactation, but decreased to control levels or very low values immediately after birth and weaning, respectively. Furthermore, the concentration of 1,25-$(OH)_2$D was inversely correlated with the concentration of calcium, suggesting that circulating 1,25-$(OH)_2$D fluctuates in concert with calcium demands during the reproductive cycle. Parathyroidectomy in lactating rats caused a 70 percent inhibition of the normally observed 1,25-$(OH)_2$D increase, indicating that parathyroid hormone, in response to changes in serum calcium, is a primary modulator of 1,25-$(OH)_2$D during lactation.*

The mineral requirements of the fetus during pregnancy and the high rates of mineral transfer to milk during lactation impose substantial drains on maternal calcium and phosphorus stores (*1*). These stores are maintained and replenished by increases in intestinal absorption of calcium and phosphate, processes known to be mediated by 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] (*2*). This active vitamin D metabolite, which also participates in calcium homeostasis, is formed in a tightly controlled fashion in the kidney (*3*) under the influence of a host of regulators including calcium (*4*), phosphate (*5*), parathyroid hormone (PTH) (*5, 6*), growth hormone (*7*), estrogen (*8*), and prolactin (*9*).

Near the end of pregnancy in humans (*10*) and late in lactation in rats (*11*), striking increases are observed in the concentration of 1,25-$(OH)_2$D in the serum. Little is known about the factors responsible for triggering the increase in circulating 1,25-$(OH)_2$D during the reproductive period. We therefore conducted experiments in rats to monitor serum 1,25-$(OH)_2$D, calcium, and phosphorus at a number of periods between late pregnancy and the termination of lactation and to determine if, during lactation, the absence of PTH might significantly alter serum 1,25-$(OH)_2$D and therefore implicate PTH as a major regulating factor.

Female rats (Holtzman) were obtained when they were 6 or 14 days pregnant, when they had litters of ten 7- to 15-day-old pups, or when they were nonlactating adults. All rats arrived in the laboratory 6 days or more before the beginning of the experiment and were maintained on a diet (*11*) consisting of 75 percent whole wheat flour, 13 percent casein, 4.4 percent fat (mostly as corn oil), 2 percent salt mixture, 1.3 percent of vitamin D–free vitamin mixture (ICN Life Sciences), and 0.8 percent $CaCO_3$, with final calcium and phosphorus contents of 0.37 and 0.32 percent, respectively. Vitamin D_3 was added at 5 I.U. per gram of diet.

Calcium, phosphorus, and 1,25-$(OH)_2$D were determined in the serum of rats at different reproductive stages (six to eight animals per group). The stages used were pregnancy (at 21 days), shortly after birth (1 to 2 days), lactation (7 to 8 days and 13 to 16 days), and postlactation (2 and 7 days after weaning). Nonlactating controls, in which lactation had been terminated 2 weeks or more, were also included. Control rats for individual reproductive groups were killed at times ranging from 6 to 20 days after receipt in the laboratory, corresponding to the time the pregnant and lactating rats were killed. Serum calcium was determined in a lanthanum chloride solution (1 percent lanthanum) by atomic absorption spectrometry with a Perkin-Elmer 305 instrument. Phosphate (as inorganic phosphorus) was determined colorimetrically (*12*). The remaining serum (3 to 5 ml per rat) was frozen (−90°C) for subsequent determinations of 1,25-$(OH)_2$D, which was measured by a radioreceptor assay with the chick intestinal cytosol-chromatin receptor system as originally described by Brumbaugh *et al.* (*13*) and modified as detailed elsewhere (*9*).

Recently, we have used 1,25-$(OH)_2$-[^{3}H]D_3 of very high specific activity (94 Ci/mmole) to increase the sensitivity of the assay to 1 pg of 1,25-$(OH)_2$D which allows analysis of individual rat serum samples. The 1,25-$(OH)_2$D was isolated from serum prior to assay either as described by Hughes *et al.* (*14*) or by recent modifications incorporating high-pressure liquid chromatography (*15*). Standard errors were calculated from analysis of variance of the data in each experiment and a one-tailed Student's t-test was used to test significance of differences between mean values. Significance of correlation coefficients was tested according to Mack (*16*).

Figure 1 indicates that a significant increase in 1,25-$(OH)_2$D occurs in late pregnancy in rats, as in humans. This in-

0036-8075/79/0629-1427$00.50/0

Table 1. Effect of parathyroidectomy on the concentrations of 1,25-$(OH)_2D$, calcium, phosphorus, and body weight in lactating rats.

Group	Number of rats	Serum calcium (mg/dl)		Serum phosphorus (mg/dl)		Body weights 48 hours after surgery (g)		Serum 1,25-$(OH)_2D$ 48 hours after surgery (pg/ml)*
		Before surgery	48 hours later	Before surgery	48 hours later	Mothers	Litters	
Nonlactating with sham operations	10	10.2 ± 0.1	9.9 ± 0.2	5.6 ± 0.4	4.4 ± 0.3	335 ± 9		39 ± 9
Lactating with sham operations	12	9.6 ± 0.1†	9.4 ± 0.2†	6.4 ± 0.3†	5.8 ± 0.3†	385 ± 8	250 ± 10	220 ± 8‡
Lactating parathyroidectomized	8	9.8 ± 0.1	4.5 ± 0.3	6.7 ± 0.4	8.4 ± 0.3	370 ± 10	235 ± 12	95 ± 9‡, §

*Average value from triplicate assays of four to six pools of serum from two to three rats each. †Significantly different ($P < .01$) from value for nonlactating group with sham operations. (Values before and after surgery were combined.) ‡Significantly different ($P < .01$) from value for nonlactating group with sham operations. §Significantly different ($P < .01$) from value for lactating groups with sham operations.

crease appears to be approximately 80 percent above the nonpregnant control levels and supports the belief that fetal demands for minerals during pregnancy lead in some way to enhance the synthesis of 1,25-$(OH)_2D$. By 1 to 2 days postpartum, 1,25-$(OH)_2D$ decreased to control levels, then increased again so that by 7 to 8 days of lactation the concentration of circulating hormone was nearly equal to that during pregnancy. The concentration of 1,25-$(OH)_2D$ remained high throughout lactation only to fall again precipitously 1 to 2 days after weaning (Fig. 1). A small but significant reduction in serum calcium was observed during the later stages of lactation, as documented previously (*11, 17*), but significant hypercalcemia and hyperphosphatemia occurred after weaning (Fig. 1). This physiological rebound phenomenon, seen also in cows (*18*), is apparently caused by the sudden absence of a lactation-associated calcium drain into the milk with continued high rate of calcium absorption and may be responsible for the low concentration of 1,25-$(OH)_2D$. Finally, by day 7 after weaning, 1,25-$(OH)_2D$ and calcium returned to control levels. These data illustrate the fluctuations in 1,25-$(OH)_2D$ that occur during the physiological changes of reproduction and emphasize the control exerted over this hormone during states of enhanced mineral need.

We also tested statistically the possibility that the concentrations of either calcium or phosphorus in the serum correlate strongly with the circulating concentration of 1,25-$(OH)_2D$. A linear regression analysis of individual 1,25-$(OH)_2D$ values against the corresponding calcium or phosphorus levels ($N = 55$) revealed a correlation coefficient of -0.6 ($P < .001$) for calcium and -0.3 ($P < .05$) for phosphorus. The strong negative correlation with calcium over a wide range of serum values supports the idea that this ion modulates the biosynthesis or degradation of 1,25-$(OH)_2D$, but does not rule out an influence of phosphate.

Since calcium is thought to regulate renal 1,25-$(OH)_2D$ production by way of alterations in circulating PTH (*2*), parathyroidectomy was performed in lactating rats in an attempt to reduce the concentration of serum 1,25-$(OH)_2D$. Two days before the experiment the parathyroid glands were removed by excision from rats suckling ten 12- or 13-day-old pups. Another group of lactating rats as well as a group of nonlactating rats of similar age were subjected to sham operations. Completeness of the parathyroidectomy (PTX) was assumed when a postoperative (48 hours) decrease in serum calcium of at least 3 mg/dl was observed. The mean decrease in serum calcium for the PTX group was actually 5.4 mg/dl in contrast to no significant change in the two groups with sham operations (Table 1). Six of the 11 rats showed some degree of tetany; three, sufficient to require their elimination from the experiment. The data in Table 1 illustrate that PTX has a marked inhibiting effect on the normal increase in serum 1,25-$(OH)_2D$ occurring in late lactation. Rats with sham operations at 12 to 13 days of lactation exhibited the characteristic hypocalcemia of lactation, a mild hyperphosphatemia not detected in the first experiment (Fig. 1) but reported previously (*11*), and a fivefold increase in serum 1,25-$(OH)_2D$ over nonlactating controls (somewhat greater than that shown in Fig. 1). The PTX group displayed the expected hypocalcemic and hyperphosphatemic responses and

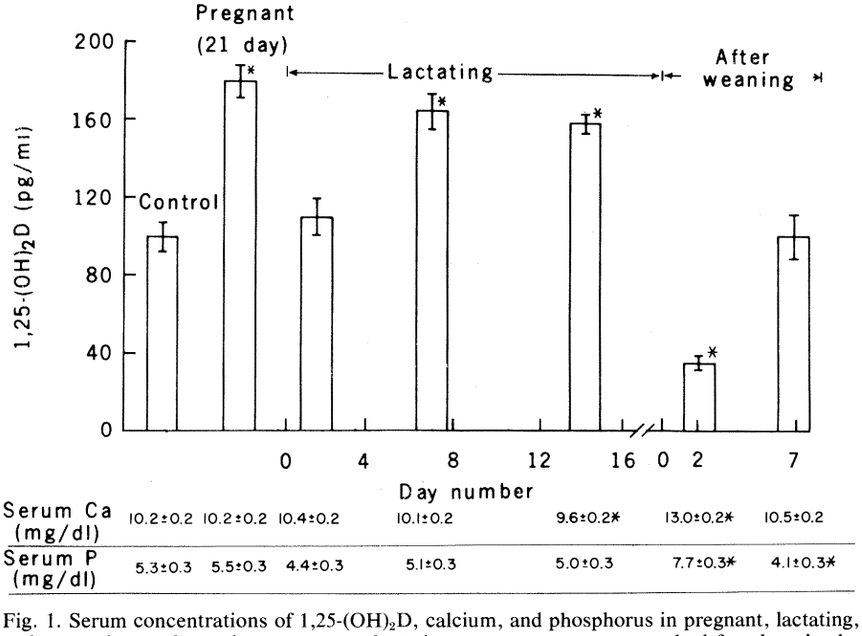

Fig. 1. Serum concentrations of 1,25-$(OH)_2D$, calcium, and phosphorus in pregnant, lactating, and weaned rats. Control rats were nonlactating, nonpregnant, age-matched female animals, three of which were bled on the same day as the 7- to 8-day lactating group; the five remaining rats were bled on the same day as the 13- to 16-day lactating group. All numbers were the average of triplicate determinations on each of seven to eight individual rat serum samples (± standard error). Values designated with an asterisk are significantly different from the control, $P < .01$.

showed a 70 percent reduction of the 1,25-$(OH)_2D$ increase that occurred in the lactating rats with sham operations. These results indicate that PTH may control 1,25-$(OH)_2D$ synthesis during lactation, as well as in other situations observed previously (*5*, *6*), although it is possible that the high phosphate concentration in the PTX group (Table 1), as well as in the group that had been weaned 2 days previously (Fig. 1) may also serve to depress the hormone. In addition, these findings do not rule out still other regulators because the PTX group retained concentrations of 1,25-$(OH)_2D_3$ that were twice that of the nonlactating group.

Since Bouillon *et al.* (*19*) have demonstrated that the serum vitamin D–binding protein, which transports 1,25-$(OH)_2D$ in the blood, does not increase in either pregnancy or lactation in the rat, the observed changes in circulating 1,25-$(OH)_2D$ (Fig. 1) could not be the result of increased plasma carrier protein synthesis. We propose that the increases in 1,25-$(OH)_2D$ in pregnancy and lactation are produced by increased biosynthesis of the hormone as catalyzed by the renal 25-hydroxyvitamin D-1-hydroxylase. This enzyme is probably stimulated by augmented calcium requirements, with the parathyroid glands acting as an intermediary to sense low serum calcium and elaborate PTH which in turn increases the activity of the 1-hydroxylase. Other evidence suggests that prolactin can also influence the 1-hydroxylase (*20*) and circulating 1,25-$(OH)_2D$ (*9*), at least in birds. Further, bromocriptine, a drug that inhibits prolactin secretion, can drastically reduce 1,25-$(OH)_2D$ concentrations in early lactation in rats, an effect that is reversed by administration of prolactin (*21*). Since serum prolactin concentrations are maximum at 2 days of lactation in rats (*22*), it is therefore probable that any effect of prolactin on 1,25-$(OH)_2D$ occurs during early lactation and that PTH is primarily responsible for the increased 1,25-$(OH)_2D$ in late lactation. A synergistic affect of PTH and prolactin is also conceivable. The physiological function of the elevated 1,25-$(OH)_2D$ concentration in lactation is probably to maintain a normal serum calcium level, since vitamin D–deprived lactating rats with reduced 1,25-$(OH)_2D$ concentrations develop severe hypocalcemia (*11*). This action of 1,25-$(OH)_2D$ may be by intestinal absorption of calcium, although direct evidence for this in lactating rats is not yet available.

In mammalian pregnancy the humoral activator of the 1-hydroxylase is also obscure, although PTH (*1*) and 1,25-$(OH)_2D$ (*18*) are both increased near term in human pregnancy, suggesting that PTH is again functioning to stimulate 1,25-$(OH)_2D$ formation. It is interesting that neither PTH (*1*) nor 1,25-$(OH)_2D$ (*23*) are increased in women with well-established lactation (6 weeks postpartum)—again pointing to the inextricable association of PTH and increased 1,25-$(OH)_2D$ in the calcium alterations of health and disease.

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24. Supported by PHS grants AM-15781 (to M.R.H.), DE-02668, and RR-05333. J.W.P. is supported by training grant GM-07239.

13 November 1978; revised 22 February 1979

Dark Anaerobic Dinitrogen Fixation by a Photosynthetic Microorganism

Abstract. *Photosynthetic purple bacteria can grow with dinitrogen gas as the sole nitrogen source under anaerobic conditions with light as the energy source. The bacterium* Rhodopseudomonas capsulata *can fix nitrogen in darkness with alternative energy conversion systems, namely, anaerobic sugar fermentation and aerobic respiration at low oxygen tension. Although growth on dinitrogen is optimal under photosynthetic conditions, the results show that reduction of dinitrogen is not obligatorily coupled to activity of the photosynthetic apparatus.*

The earliest studies on dinitrogen (N_2) fixation by photosynthetic purple bacteria capable of growing in several alternative modes indicated that N_2 reduction occurred only under anaerobic photosynthetic conditions (*1*, *2*). No growth was observed under an atmosphere of air with N_2 as sole nitrogen source (*2*), presumably because of the inhibitory effects of molecular oxygen on nitrogenase synthesis and activity (*3*). A recent investigation on the characteristics of N_2 fixation by the purple bacterium *Rhodopseudomonas capsulata* (*4*) suggested that in this organism N_2 fixation is dependent on the availability of a reductant uniquely produced by the photosynthetic apparatus. We report experiments demonstrating that *R. capsulata* can grow on N_2 as sole nitrogen source anaerobically in darkness; thus, a "photoreductant" is not obligatory.

Rhodopseudomonas capsulata grows anaerobically in darkness on N_2 in a completely defined medium with fructose as the sole source of carbon, energy, and reducing power (Fig. 1). The doubling time is approximately 12 hours,

0036-8075/79/0629-1429$00.50/0