to the origin of these inbred strains. In particular, the C3H/HeJ strain was originally derived by Strong from a cross of a Bagg albino ♀ × DBA ♂, and the BALB/c strain was originally the Bagg albino stock (*17*). Therefore, the C3H/HeJ strain may have acquired the locus for the BALB/c virus from the initial albino cross. This can be further assessed by examining the current DBA mice, which also appear to have a single AKR-type locus for MuLV (*1*). In contrast to the BALB/c and C3H/HeJ strains, the C57BL/6 strain has a completely independent origin. These observations suggest that endogenous ecotropic viruses probably became integrated in the inbred strains of mice prior to the derivation of these strains (≃1900) and certainly within the relatively recent evolutionary history of mice. As more viral loci are mapped it will be interesting to see the extent of diversity. If, in fact, there is a great diversity, the site of viral integration may actually be an important marker for the probable origin of strains of mice of unknown or questionable derivation.

The presence of the BALB/c, C3H/HeJ viral locus on chromosome 5 is of interest because the cell receptor for the virus is also on chromosome 5 (*18*). The presence of both loci on chromosome 5 appears to be fortuitous because, unlike the C3H/HeJ C-type virus, the cell receptor is common to all the mouse strains examined including the NIH Swiss strain which genetically lacks the virus.

Although the BALB/c and C3H/HeJ loci are probably allelic and code for very similar if not identical viruses, the phenotypes of expression are quite distinct; the C3H/HeJ virus is expressed early in life, whereas the BALB/c virus is only expressed late in life. This observation argues against the possibility that the site of integration plays a major role in determining the phenotype of expression. However, since the phenotype for expression of the C3H/HeJ virus segregates with the structural gene for the virus, it appears that there probably are closely linked functions which regulate expression in vivo. Nevertheless, as new techniques are developed for examining the integrated structure of the virus, these observations will be useful in studying the cellular controls of viral expression.

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Vitamin D Deficiency and Reproduction in Rats

Abstract. *Female weanling rats from a colony maintained on a diet low in vitamin D were raised on a diet that was deficient in vitamin D but was otherwise adequate. Vitamin D deficiency was confirmed in the rats by hypocalcemia and the absence of vitamin D metabolites in blood. These females gave birth to litters that were slightly smaller than control litters from females maintained on a vitamin D–containing diet. The pups from the vitamin D–deficient mothers appeared normal throughout lactation, and at weaning had normal concentrations of calcium and phosphate in the plasma. These results indicate that vitamin D and its metabolites are not necessary for reproduction and fetal development in the rat.*

The importance of vitamin D in calcium and phosphate metabolism is well known (*1, 2*). Vitamin D is converted to a hormone, 1,25-dihydroxyvitamin D [1,25-$(OH)_2D_3$], before it can function in the regulation of calcium and phosphorus metabolism (*3–6*). Recent studies have shown that vitamin D is required for normal embryonic development in the chicken (*7*). Toverud (*8*), however, has found evidence that vitamin D is not essential for lactation nor for maintaining a normal level of calcium in the milk of lactating rats (*8*). We have therefore investigated whether or not mammals kept on a strict vitamin D–deficient diet from weaning can reproduce and maintain their offspring.

Female Holtzman rats were obtained as weanlings from a colony kept on a diet low in vitamin D. The weanlings were divided into two groups: both groups received a vitamin D–deficient diet that contained 0.47 percent calcium and 0.3 percent phosphorus (*9*), and one of these groups received 25 units of vitamin D_3 (orally) per day. At no time were any of the animals exposed to ultraviolet radiation, thus the possibility of vitamin D being produced endogenously in the skin was eliminated. Each group was maintained on its respective diet throughout the experiment, and at age 110 days the animals were mated with normal, vitamin D–replete males.

The number of females becoming pregnant, reaching term, and giving birth to normal-appearing offspring from each group was similar. When the newborn pups reached an age of 23 days postpartum they were weaned. At this time, four to five pups were taken randomly from each litter and killed. The remaining pups were placed in individual cages and fed the same diet that their respective mothers had received. Mothers and nonmated females from the vitamin D–deficient and vitamin D–replete groups were also killed. Plasma samples were analyzed for the vitamin D metabolites 25-hydroxyvitamin D_3 (25-OH-D_3) and 1,25-$(OH)_2D_3$ by previously established methods (*10, 11*). Plasma calcium concentration was determined by diluting 0.1-ml samples of plasma with 1.9 ml of 0.1 percent aqueous $LaCl_3$ and measur-

Table 1. Plasma concentrations of 25-OH-D_3 and 1,25-$(OH)_2D_3$ in vitamin D–deficient (−D) and vitamin D–replete (+D) rats. Data are expressed as means ± standard error (S.E.). The numbers of rats in each group is given in parentheses.

Group	25-OH-D_3 (ng/ml)	1,25-$(OH)_2D_3$ (pg/ml)
+D	21 ± 6 (N = 6)	25 ± 5 (N = 3)
−D	0* (N = 6)	0† (N = 3)

*Not detectable, < 0.5 ng/ml. †Not detectable, < 5 pg/ml.

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Table 2. Concentrations of calcium and phosphate in the plasma of vitamin D-deficient (−D) and vitamin D-replete (+D) females and mothers at time of weaning (age of pups, 23 days). Data are expressed as means ± S.E. The number of rats in each group is given in parentheses.

Group	Calcium (mg/100 ml)	Phosphate (mg/100 ml)
Nonmated female +D	9.9 ± 0.2 (N = 5)	6.8 ± 0.4 (N = 4)
Mother +D	8.8 ± 0.2* (N = 3)	5.9 ± 0.7† (N = 3)
Nonmated female −D	5.6 ± 0.4 (N = 5)	6.6 ± 0.5 (N = 5)
Mother −D	4.1 ± 0.4‡ (N = 4)	4.6 ± 0.4§ (N = 4)

*P < .025, for +D mother compared with +D nonmated female. †Not significantly different from +D nonmated female. ‡P < .05 for −D mother compared with −D unmated female. §P < .025 for −D mother compared with −D unmated female.

Table 3. Concentrations of calcium and phosphate (as milligrams per 100 ml) in the plasma at time of weaning (23 days postpartum) and 21 days after weaning of rat pups from vitamin D-deficient (−D) and vitamin D-replete (+D) mothers. Data are expressed as means ± S.E. The number of litters analyzed is given in parentheses. Three to five animals were taken randomly from each litter and the mean calcium and phosphate concentrations in the plasma were measured. These means were in turn used to calculate the overall mean (reported value) for a given nutritional state.

Group	At weaning		21 days after weaning	
	Calcium	Phosphate	Calcium	Phosphate
+D	9.0 ± 0.1 (N = 3)	10.4 ± 0.3 (N = 3)	10.2 ± 0.2 (N = 3)	8.7 ± 0.6 (N = 3)
−D	9.3 ± 0.6* (N = 4)	9.3 ± 1.1* (N = 4)	7.2 ± 0.2† (N = 3)	7.4 ± 0.2* (N = 3)

*Not significantly different (Student's t-test) from +D litters. †P < .001, for +D mothers compared with −D mothers (Student's t-test).

ing the calcium concentration by atomic absorption spectroscopy. Phosphate concentrations in the plasma were measured by the method of Chen *et al.* (*12*).

After approximately 5 months, no detectable 25-OH-D_3 or 1,25-$(OH)_2D_3$ was found in the plasma of rats fed the vitamin D-deficient diet (Table 1). Plasma from vitamin D-replete rats contained, per milliliter, 21 ng and 25 pg of 25-OH-D_3 and 1,25-$(OH)_2D_3$, respectively. These results verify that the rats fed the vitamin D-deficient diet were, in fact, vitamin D-deficient. However, because of the finite detectability limits for 1,25-$(OH)_2D_3$, it is impossible to say that these animals were absolutely vitamin D-deficient. Nevertheless, we can say that with 1,25-$(OH)_2D_3$ concentrations in the plasma of less than 5 pg/ml and plasma calcium concentrations on the order of 5.6 mg/100 ml that these animals were, as far as we can determine, more deficient than other animals in previously published experiments. Metabolite levels were not measured in the experiments of Toverud (*8*), and in his vitamin D-deficient group the serum calcium concentrations were of the order of 8.5 mg/100 ml.

The concentrations of calcium and phosphate in the plasma of vitamin D-deficient and vitamin D-replete animals are given in Table 2. Not only did pregnancy and lactation result in a significant decrease in plasma calcium and, in the case of the vitamin D-deficient animals, a significant decrease in plasma phosphate, but also there was a general and dramatic decrease in plasma calcium in the vitamin D-deficient animals. Plasma phosphate was unaffected by vitamin D deficiency except in the case of the vitamin D-deficient mothers.

The mean weight (± standard error) of vitamin D-deficient females at age 5 months was 251 ± 13 g, whereas the mean weight of vitamin D-replete females was 305 ± 9 g. Litter sizes in the vitamin D-deficient animals averaged 8 ± 0.6, whereas in vitamin D-replete animals they averaged 12 ± 0.8.

In general, pups born to vitamin D-deficient and vitamin D-replete mothers appeared visually similar at birth and throughout lactation. No difference in pup weights was noted at 24 hours postpartum between the two groups. Pup weights at weaning were also similar.

Plasma concentrations of calcium and phosphate in the pups at weaning and at 21 days after weaning are given in Table 3. Pups from vitamin D-deficient mothers had plasma clacium and phosphate concentrations similar to those of pups from vitamin D-replete mothers at weaning. Twenty-one days after weaning, however, calcium and phosphate concentrations in the plasma of vitamin D-deficient pups had decreased to 7.2 ± 0.2 and 7.4 ± 0.2 mg/100 ml, respectively, which, in the case of calcium, represents a significant decrease when compared to vitamin D-replete pups (10.2 ± 0.2 mg/100 ml). The lack of hypocalcemia in pups from vitamin D-deficient mothers during lactation suggests a more or less normal intestinal absorption of calcium in the pup. This may result from the enhancement of calcium transport in the small intestine by lactose (*13*). The source of calcium for pup growth during lactation is apparently the bone of mothers. Preliminary results from our laboratory indicate that in both vitamin D-replete and vitamin D-deficient mothers, bone calcium is reduced during lactation.

These findings demonstrate that vitamin D is not required for reproduction in the rat. Pups born to vitamin D-deficient mothers appear grossly normal at birth and remain so throughout lactation. Weight gain is normal and, at the time of weaning, plasma calcium and phosphate concentrations are similar to those seen in vitamin D-replete pups. However, pups from vitamin D-deficient mothers may not have a normal calcium homeostasis during nursing, because we found that the degree of bone calcification in weanling pups from vitamin D-deficient mothers was less than in pups from vitamin D-replete mothers. Our results do suggest, however, that vitamin D is not essential for calcium mobilization in the female during pregnancy and lactation and further, that vitamin D is not necessary to maintain a normal plasma calcium concentration in pups during lactation.

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