Reduced Survival of Neonates due to Vitamin A Deficiency during Pregnancy in the Guinea Pig (43224)

J. APGAR,* A. DULIN,† T. KRAMER,†,[1] AND J. C. SMITH†

U.S. Plant, Soil and Nutrition Laboratory, Ithaca, New York 14853-0331 and Vitamin and Mineral Nutrition Laboratory,† U.S. Department of Agriculture, ARS, Beltsville Human Nutrition Research Center, Beltsville, Maryland 20705*

Abstract. **Neonatal vitamin A stores are limited even in well-nourished full-term infants and are yet smaller in the premature infant. The object of this experiment was to determine whether vitamin A deficiency could be induced in pregnant guinea pigs and, if so, whether it would affect vitamin A status of the neonate. Adult (600 g) guinea pigs were fed a casein-agar diet that was vitamin A deficient (AD). Controls (vitamin A adequate) were orally dosed weekly with 2 mg of retinyl palmitate. Weight gains of dams and birth weights of neonates did not differ. No external signs of deficiency were observed. Six of eight AD and seven of eight vitamin A-adequate dams carried pregnancy to term (≥Day 64). One AD dam died during delivery. Liver retinol concentrations were below the detection limit (<3 μg/g) for all AD neonates and dams and in postpartum serum of AD dams. Of neonates born ≥Day 64, 15 of 18 AD were dead or moribund compared with 4 of 22 vitamin A adequate. The unexpectedly severe effect on the neonate indicates that the guinea pig will be a sensitive model for investigating the affect of poor maternal vitamin A status on neonatal vitamin A-dependent functions. However, a less severe maternal deprivation should be used for such studies.**

[P.S.E.B.M. 1991, Vol 197]

Although overt vitamin A deficiency is considered to be rare in the United States, it is a major nutritional problem in some parts of the non-industrialized world (1). Infants appear to be particularly vulnerable since liver stores of vitamin A are limited even in well-nourished full-term infants compared with concentrations later in infancy (2), and concentrations in premature infants are even less than those in full-term infants (3). Inadequate liver vitamin A has been suggested to be related to the respiratory difficulties of the premature infant since 40% of lung tissue consists of epithelial cells, and vitamin A is essential to the health of epithelial tissue (3). Vitamin A, in common with other nutrients, is also essential for the optimal response of the immune system (4). To determine whether marginal maternal vitamin A status will affect the neonate, we chose the guinea pig as a model for several reasons: (i) The stage of maturity of the neonatal immune system more closely resembles that of the human infant than does that of the rat or mouse (5). (ii) The transfer of maternal antibodies through the placenta and the immunoglobulin composition of colostrum and milk are also similar to those of the human (6). (iii) The guinea pig is easier to milk than other laboratory animals. (iv) The pregnant guinea pig is relatively large with a long gestation (68 days), which permits serial blood sampling of the same individual during a developing deficiency. (v) It has a higher percentage of body fat in the neonate than other laboratory animals, another characteristic that it shares with the human (7).

This experiment was designed to test whether female guinea pigs fed a vitamin A-deficient diet after reaching breeding age would be able to maintain pregnancy and still be sufficiently depleted by the end of gestation to affect the vitamin A status of the neonate. Since rats can store sufficient vitamin A during periods of ample supply to last throughout their lifetime (8), studies of vitamin A deficiency during pregnancy in the rat have required raising the animals on diets with low vitamin A content (9, 10). The only report we found of

[1] To whom requests for reprints should be addressed at USDA, ARS, BHNRC, VMNL, Bldg. 307, Rm 116, BARC-East, Beltsville, MD 20705.

Received September 10, 1990. [P.S.E.B.M. 1991, Vol 197]
Accepted January 10, 1991.

0037-9727/91/1971-0056$3.00/0

vitamin A deficiency in the pregnant guinea pig was that of McHowell *et al.* (11), in which guinea pigs were fed from weaning a vitamin A-deficient diet supplemented with retinoic acid. The females failed to maintain pregnancy, discharging placental remnants approximately 3 weeks after mating. Because of the ability of the rat to store large amounts of vitamin A and the lack of information on the guinea pig, we tested whether a deficiency could be induced in the pregnant guinea pig without feeding a deficient diet during growth.

Materials and Methods

Adult guinea pigs weighing approximately 600 g were purchased commercially (Camm Research Lab Animals,[2] Wayne, NJ). The experimental diet was a casein-based agar diet (Table I) to which no vitamin A was added. To change the adult animals from the commercial pelleted ration to the purified diet, diced agar diet was mixed with decreasing amounts of the commercial pelleted feed. Since the guinea pig does not manipulate the diet with its forepaws as the rat does (12), it is difficult for the animal to eat a powdered diet especially as it becomes compacted. However, mixing the diet 1:1 with 2% agar permits feeding the diet as a solid block once the animals have adapted to the diet. The blocks were frozen until needed. Most of the animals had adapted to the purified diet at the end of 2 weeks.

All animals were fed the vitamin A-deficient diet (Table I). The vitamin A-adequate (AA) controls were dosed orally with 2 mg of retinyl palmitate once a week after mating or after the first missed estrus if a copulation plug was not found. The dose was chosen because it provided similar retinol equivalents to that provided by consuming a diet containing 6 mg of vitamin A/kg diet, assuming a daily intake of 30 g of diet. This concentration, 6 mg/kg, has been reported to permit storage of vitamin A in the liver of the guinea pig (13). Vitamin A-deficient (AD) guinea pigs were not treated. Because the diet was also used for an experiment involving low zinc intake, zinc was not included in the mineral mix. All animals therefore received adequate zinc in the drinking water, 15 mg of zinc/liter as zinc acetate.

Table I. Vitamin A-Deficient Diet

	(g/kg)
Casein (EDTA-treated)	300
Glucose monohydrate	150
Corn starch	170
Cellulose	150
Corn oil	100
Mineral mix[a]	92
Vitamin mix[b]	38

[a] Mineral mix supplied per kg diet: in g—$Na_2HPO_4 \cdot 7H_2O$, 28; CH_3COOK, 24; $CaCO_3$, 12.9; $CaHPO_4$, 7.4; $MgSO_4 \cdot 7H_2O$, 4.9; MgO, 4.4; $MgCO_3$, 0.9; KCl, 4; NaCl, 2.5; $FeSO_4 \cdot 7H_2O$, 2.04; $MnSO_4 \cdot H_2O$ 0.71; in mg—$CuSO_4 \cdot 5H_2O$, 50; KIO_3, 34; $CoCl_2 \cdot 6H_2O$, 27.

[b] Vitamin mix supplied per kg diet: in g—ascorbic acid, 4; inositol, 4; choline, 3.1; niacin, 0.4; in mg—calcium pantothenate, 60; riboflavin, 30; thiamin·HCl, 30; pyridoxine·HCl, 13.5; biotin, 12.6; folic acid, 12; menadione, 4.6; in μg—vitamin B_{12}, 20; in IU—vitamin D, 4400; vitamin E, 198.

[2] Mention of a proprietary product does not imply its approval to the exclusion of other products that may be suitable.

Breeding was started at the end of the 2-week adaptation period. Females were individually caged with a male at the time of expected estrus. Because of the limited number of males available and the relatively long estrus cycle (17 days), breeding extended over a 6-week period. Six of the AD animals mated within 10 days of the start of breeding; one after 20 days, and the last one after 6 weeks. All AA females mated within 6 weeks of the start of breeding. One for which a copulation plug was not found aborted shortly after vitamin A dosing was started. (Dosing had begun at the first missed estrus.) Vitamin A dosing was continued, and the female mated again at the next estrus.

Pregnant animals were weighed daily. Starting at Day 50 they were checked for delivery at least three times daily. Neonates were weighed and liver dissected from dead or moribund animals. Litter size was restricted to not more than three; any additional pups were killed and blood and liver were collected. Females were killed after delivery if there were no viable pups; otherwise, after 2 weeks of lactation.

Saponified (14) liver samples were analyzed for vitamin A according to the trifluoroacetic acid method of Neeld and Pearson (15). Values in the text and Table II are mean ± SE. The difference between treatments for the number of dead or moribund pups was subjected to chi-square analysis (16). Differences between the detection limit and mean liver retinol concentrations for the vitamin A-adequate treatment was analyzed by Student's *t* test (16).

Results and Discussion

Weight gains of dams did not differ (Table II); nor were any external signs of vitamin A deficiency observed. Mean birth weight and litter size also did not differ (Table II). The slightly lower mean birth weight of AD animals is due to two litters that were delivered prematurely, Days 57 and 59. The remaining six AD and seven of eight AA females carried pregnancy to at least Day 64, which is about the earliest time at which neonates survive (Table II), although in this experiment the AA female that delivered on Day 64 had one dead and three moribund pups. One AD female was unable to deliver and died on Day 70; it was the last AD female to mate and had been on the deficient diet for 6 weeks prior to breeding. Vitamin A deficiency was reported in the 1930s to interfere with parturition in the rat (17).

Table II. Effect of Vitamin A-Deficient Diet on Outcome of Pregnancy in the Guinea Pig

	Treatment	
	Vitamin A-deficient	Vitamin A-adequate
No. of females	8	8
Female weight (g)		
Day 1[a]	597 ± 12	647 ± 24
Day of delivery	1029 ± 36	1069 ± 41
Post partum	655 ± 22	693 ± 23
Litter size	3.1 ± 0.2	3.2 ± 0.2
No. pregnant ≥Day 64	6	7
No. pups carried to term (≥Day 64)	18	22
No. full-term pups dead or moribund[b]	15	4
Birthweight (g)	97 ± 10	102 ± 8
Liver retinol (μg/g wet weight)		
Maternal[c]	≤3.0	65 ± 18
Neonatal[c]	≤3.0	7.8 ± 2

[a] Values are mean ± SE.
[b] Treatments differ significantly ($P < .001$).
[c] Mean of vitamin A adequate differs significantly from detection limit, 3 μg/g (*t* test: maternal, $P < 0.01$; neonatal, $P < 0.05$).

In view of the normal weight gains of the AD dams and the normal birth weights of their pups, the poor viability of the pups was unexpected (Table II). Of the 18 neonates that were carried to term (≥Day 64), only five were alive at birth; two of those were moribund. Of the 22 AA pups delivered on Day 64 or later, 21 were born alive, 3 of which were too weak to survive.

That the reduced viability of AD neonates was due to poor vitamin A status is supported by the low concentration of retinol in both maternal and neonatal liver (Table II). The concentration of retinol in the livers of all AD neonates and dams was below the detection limit, 3 μg/g, equivalent to 0.01 μmol/g. The low concentration in the dams represents a considerable decrease in liver stores since the concentration in livers of females of similar body weight purchased from the same supplier and fed a commercial guinea pig diet was 190 ± 31 μg/g.

The AA neonate had much less retinol in the liver than did the dam, approximately 12% of the maternal concentration. Maternal liver retinol in the rat has also been reported to be considerably higher than that in the fetus if vitamin A intake exceeds the requirement (2).

Since the concentration of vitamin A in neonatal liver of humans as well as animals is low, there does not appear to be much of a safety margin. If the mother is deficient, the level may be further decreased. How much the vitamin A concentration in the neonatal liver can decrease without affecting survival is not clear.

Retinol was also below the detection limit in the postpartum plasma of AD dams (data not shown). There was only one AD litter with viable neonates; retinol in plasma of these neonates was also below the detection limit.

We therefore conclude that Vitamin A deficiency can be induced in the pregnant guinea pig. Unlike the rat, it is not necessary to feed the guinea pigs a vitamin A-deficient diet from weaning; the deficiency can be induced in adults that have received a normal diet. Maternal weight gain is not affected. Neonatal survival is severely reduced in spite of normal gestation length and normal birth weights. Adjustment of the vitamin A intake of the pregnant guinea pig should produce viable neonates with low A stores, suitable for studying the effect of poor maternal vitamin A status on the fetal immune system and on other parameters.

1. Underwood BA. Vitamin A prophylaxis programs in developing countries: Past experiences and future prospects. Nutr Rev **48**:265–274, 1990.
2. Wallingford JC, Underwood BA. Vitamin A deficiency in pregnancy, lactation, and the nursing child. In: Bauernfeind JC, Ed. Vitamin A Deficiency and Its Control. New York: Academic Press, pp101–152, 1986.
3. Zachman RD. Retinol (vitamin A) and the neonate: Special problems of the human premature infant. Am J Clin Nutr **50**:413–424, 1989.
4. Watson RR, Rybski JA. Immunological response modification by vitamin A and other retinoids. In: Chandra RK, Ed. Nutrition and Immunology. New York: Alan R. Liss, pp87–99, 1988.
5. Sisk DB. Physiology. In: Wagner JE, Manning PJ, Eds. The Biology of the Guinea Pig. New York: Academic Press, pp63–98, 1976.
6. Ali HM, Scott R, Toms GL. Effect of foster feeding and bottle feeding expressed breast-milk on susceptibility of guinea-pig infants to influenza virus. Br J Exp Pathol **70**:183–191, 1989.
7. Widdowson EM. Chemical composition of newborn mammals. Nature **166**:626–627, 1950.
8. Moore T. Effects of vitamin A deficiency in animals. In: Sebrell WH Jr, Harris RS, Eds. The Vitamins. New York: Academic Press, pp245–266, 1967.
9. Rogers WE Jr, Bieri JG. Vitamin A deficiency in the rat prior to weaning. Proc Soc Exp Biol Med **132**:622–623, 1969.
10. Takahashi YI, Smith JE, Winick M, Goodman DS. Vitamin A deficiency and fetal growth and development in the rat. J Nutr **105**:1299–1310, 1975.
11. McHowell J, Thompson JN, Pitt GAJ. Changes in the tissues of guinea-pigs fed on a diet free from vitamin A but containing methyl retinoate. Br J Nutr **21**:37–44, 1967.
12. Hirsch E. Some determinants of intake and patterns of feeding in the guinea pig. Physiol Behav **11**:687–704, 1973.
13. Gil A, Briggs GM, Typpo J, MacKinney G. Vitamin A requirement of the guinea pig. J Nutr **96**:359–362, 1968.
14. Bieri JG. Chromatography of tocopherols. In: Marinetti GV, Ed. Lipid Chromatographic Analysis. New York: Marcel Dekker, Vol **2**: pp459–478, 1968.
15. Neeld JB Jr, Pearson WN. Macro- and micromethods for determination of serum vitamin A using trifluoroacetic acid. J Nutr **79**:454–462, 1963.
16. Snedecor GW. Statistical Methods. 5th Ed. Ames, IA: Iowa State College Press, 1956.
17. Mason KE. Prolonged gestation and difficult parturition in the rat as a result of vitamin A deficiency. Am J Anat **57**:303–313, 1935.