# Vitamin A Deficiency in the Rat Prior to Weaning (34273)

W. E. Rogers, Jr.[1] and J. G. Bieri

*Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014*

During the last few years increasing use has been made of rats maintained on retinoic acid for studies of vitamin A deficiency (1). Since this form of the vitamin disappears rapidly from the animal when supplementation is stopped, the variable stress from infection and inanition which occurs in conventional vitamin A deficiency is largely avoided. Through the use of retinoic acid it is possible to produce animals which become vitamin A deficient prior to weaning and which are suitable for studies of the requirement of the vitamin in growth and development.

*Materials and Methods.* Female Sprague-Dawley rats with 10-day old litters were fed vitamin A deficient diet R-9 (2). The percentage composition of the diet was: vitamin-free casein, 22; salt mixture, 6; cottonseed oil, 4; vitamin mixture (without vitamin A), 2; and sucrose, 66. Female weanling rats from these litters were fed this diet for 18 days at the end of which time they were given 20 μg of retinoic acid in 5 drops of cottonseed oil placed on the diet each day. When the animals became sexually mature, about 5 weeks postweaning, they were mated with vitamin A normal males. Appearance of sperm in the vaginal smear or sperm plugs in the trays were used to establish the first day of pregnancy. During pregnancy and lactation the retinoic acid supplement was increased to 50 μg/day. In addition, from the ninth through the twenty-first day of pregnancy a supplement of 1 μg of retinyl acetate in oil was given orally. On the eighteenth day of pregnancy the females were transferred to individual plastic cages containing wood shavings where they were kept until the young were weaned.

*Results.* During first pregnancies of 30 rats parturition occurred on the twenty-second or twenty-third day and the litter size averaged 9.2, indicating that the supplement of 1 μg/day of retinyl acetate was adequate. After resting 3 weeks the females could produce a second litter of vitamin A-low rats, but third pregnancies were not as successful unless the dose of retinyl acetate was doubled. During the first days of life the rats nursed energetically and obtained milk. Subsequently they exhibited varying effects of vitamin A deficiency that depended on whether deficiency developed in the first or second week of life.

When symptoms developed in the first week the rats exhibited progressive weakness, distension of the abdomen and narrowing of the thorax. At death, usually by the twelfth day of life, the intestines were distended by liquid and gas as in older, conventionally vitamin A deficient rats. When retinyl acetate or retinoic acid (5 μg/day orally in a drop of oil) was given to the young rats before weakness became pronounced, survival was almost 100% and no abnormalities developed.

In other litters the rats maintained good appetites and remained relatively strong into the second week of life. At about the ninth to eleventh day these rats began to appear malnourished, had a yellowish cast to their skin and exhibited trembling and mild incoordination. In the next few days crippling of the front legs was manifested. The paws and toes usually curled and the young rats walked stiff-legged on the under-turned paws. In other cases, there was complete loss of

[1] Initial work on this problem was done in the Department of Animal Sciences, University of Illinois, Urbana and was supported by USPHS Grant GM-00653-06. A preliminary report was presented at the Federation of American Societies for Experimental Biology meeting, April 16-21, 1967, Chicago, Ill.

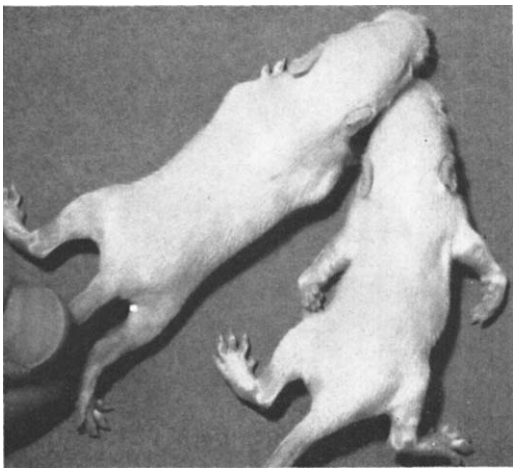

FIG. 1. Litter-mate rats on thirteenth day of life. Offspring of dam supported with retinoic acid continuously and from ninth to twentieth day of pregnancy with retinyl acetate, 1 μg/day. Young rats dosed from ninth day of life with either 5 μg of retinoic acid in droplet of cottonseed oil per day (left) or cottonseed oil (right).

use of the front legs and the rats pushed themselves along on their thorax while their forelegs dragged from the shoulder (Fig. 1). The stance of the hind legs became spread but these limbs and the rest of the muscles of the animals remained relatively strong. Eyes opened regularly at about the fourteenth day of life and specks of porphyrin pigment began to appear around the nose and eyes about the seventeenth day. If the young rats were able to obtain retinoic acid-supplemented diet from the mother's food cup, or if hand supplementation was started, the crippling disappeared. Without supplementation the rats became weaker and died, usually with distended intestines as described above. The time required for recovery was related to the length of time that the rats had been crippled. In young rats (*e.g.*, fourteenth day of life), retinoic acid supplementation restored walking function in 2–4 days.

It was found that normal appearing rats deficient in retinyl ester at weaning could be obtained consistently if the dams were supported during pregnancy by the above procedure and the young rats were supplemented, starting at the fifth day of life, with retinoic acid. Growth stopped quickly when retinoic acid was removed from the diet of weanling rats prepared in this fashion. A typical response is shown in Fig. 2 where growth is seen to stop 4 days after removal of the vitamin from the diet and to recommence promptly upon dosing the rats with 5 μg of retinoic acid in oil per day. By restricting vitamin A acid again a second cycle of weight changes followed but the time required for a decline in weight was longer and the response of the rats was more variable.

*Discussion.* Incoordination and paralysis due to vitamin A deficiency has usually been explained by either a disproportionate growth of the central nervous system and surrounding bone or by increased cerebrospinal fluid pressure (3, 4). Paralysis has generally been observed only in the hind legs of rats, but Mayer and Krehl (5) observed an involvement of the front legs in vitamin A deficient rats which was cured by dosing with ascorbic acid. We found ascorbic acid to be without effect whereas retinoic acid always produced cures.

The crippling reported here is interesting with regard to the hypothesis that this condition is due to pinching of nerve tissue by

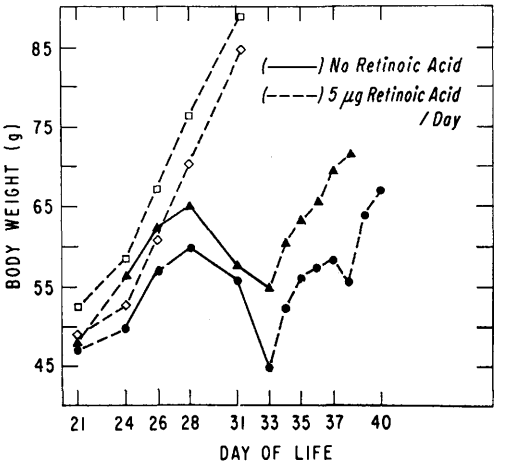


FIG. 2. Typical growth response to retinoic acid of weanling, littermate rats having low body stores of retinyl esters. Curves are for individual rats. Rats were prepared as described in text and weaned to retinoic acid-containing diet on twenty-first day of life. Retinoic acid was removed from the diet of two rats on the twenty-fourth day of life and restored on the thirty-third day of life.

bone. The fact that the paralysis is reversed in 2–4 days in its early stage would appear to suggest a more rapid process than bone remodeling. An alternative hypothesis is that nerve tissue has a direct requirement for vitamin A. Takeshita and Ko (6) reported that in the vitamin A-deficient rats changes were observed mainly in the sensory nerve endings. These investigators thought that nerve degeneration then progressed retrogradely up the sensory fibers toward the central nervous system. Also Krishnamurthy *et al.* (7) noted that chicks supported with a daily dose of retinoic acid showed ataxia each day about 20–24 hr after the supplement was given.

It has been well authenticated that retinoic acid disappears rapidly after administration to the rat. Supporting rats on retinoic acid provides a unique means of maintaining them available for the study of certain aspects of vitamin A deficiency. When the deficient condition is required, it is necessary only to remove the supplement from the test diet and in approximately 2 days the rats are free of vitamin A. Thompson *et al.* (1) reported that pregnancy could be maintained in rats supported with retinoic acid if they were given an additional supplement of very small amounts of retinyl esters. They interpreted the failure of many of the young to survive as being due to an inherent weakness resulting from the nutritional treatment of the dam during pregnancy. It seems likely that this weakness is due to lack of transport of retinoic acid to the young rats through the dam's milk for if they are hand supplemented with retinoic acid they survive. The advantages of this procedure over conventional methods of producing vitamin A deficiency are chiefly in the young age of the experimental animals and the minimizing of side-effects of deficiency such as infection and prolonged inanition.

*Summary.* Female rats maintained on retinoic acid and given a supplement of 1 μg of retinyl acetate during part of pregnancy delivered normal offspring that became vitamin A deficient in the first 2 weeks of life. If the rats were not supplemented with vitamin A, reversible crippling of the front legs occurred frequently in the second week of life. If retinoic acid supplementation was started a few days after birth, the rats developed normally but their growth would stop in 4 days whenever the supplement was removed from the diet. Growth resumed promptly when retinoic acid was again supplied.

The careful assistance of Mrs. Mary Sue Brewer in caring for the young rats is gratefully acknowledged.

1. Thompson, J. N., Howell, J. McC., and Pitt, G., A. J., Proc. Roy. Soc. (London) Ser. B **159**, 510 (1963-1964).

2. Bieri, J. G. and Prival, E. L., J. Nutr. **89**, 55 (1966).

3. Moore, T., "Vitamin A," pp. 314–328. Elsevier, Amsterdam (1957).

4. Howell, J. McC. and Thompson, J. N., Brit. J. Exptl. Pathol. **48**, 450 (1967).

5. Mayer, J. and Krehl, W. A., Arch. Biochem. **16**, 313 (1948).

6. Takeshita, T. and Ko, J., Bitamin **31**, 50 (1965).

7. Krishnamurthy, S., Bieri, J. G., and Andrews, E. L., J. Nutr. **79**, 503 (1963).

Received March 18, 1969, P.S.E.B.M., 1969, Vol. 132.