Effects of Prolonged Exposure to Cold on the Vitamin A Requirement of the Rat.* (17981)

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Available data indicate that requirements for a number of nutrients are markedly increased under conditions of low environmental temperature. This is particularly true for some of the water-soluble vitamins. An increased requirement for thiamine(1,2), pyridoxine(3) and ascorbic acid(4) has been demonstrated following prolonged exposure to low environmental temperatures. In the present communication data are presented on the effects of low environmental temperature on the vitamin A requirement of the rat. Animals were fed purified rations deficient in vit. A and their rate of depletion and length of survival determined under cold room and room temperature conditions.

Procedure and results. The vit. A-free ration employed in the present experiment consisted of sucrose, 60%; casein,† 25%; salt mixture,‡ 5%; and cottonseed oil (Wesson), 10%. To each kg of the above were added the following synthetic vitamins: thiamine hydrochloride, 40 mg; riboflavin, 40 mg; pyridoxine hydrochloride, 40 mg; calcium pantothenate, 80 mg; nicotinic acid, 60 mg; ascorbic acid, 100 mg; biotin, 5 mg; folic acid, 10 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; vit. B_{12},§ 60 γ; 2-methylnaphthoquinone, 10 mg; vit. D,‖ 400 U.S.P. units; and choline chloride, 2 g. Each rat also received once weekly 4.5 mg of alpha-tocopherol acetate. The animals employed in the present experiment were male rats of the University of Southern California strain in which the dietary regime of the parents was such as to make the young rats satisfactory for bioassay studies for vit. A. Parents were maintained for at least 2 months previous to breeding and for 14 days after the birth of the litter on Sherman diet B(5) without addition of supplementary lettuce or meat. Litters were cut to 7 at 3 days of age, and on the 14th day mothers and litters were placed on a vit. A-low diet.¶ At 26 days of age the young rats were placed either on the vit. A-free diet indicated above or a similar ration supplemented with 6000 U.S.P. units of vit. A per kg of diet.** Tests were conducted (1) with animals kept continuously in a large walk-in refrigerator at a temperature of 2 ± 1.5°C and (2) under standard laboratory conditions at an average temperature of approximately 23 ± 2°C. The dietary groups consisted of 12 rats each in the room temperature series and 16 animals per group in the cold room series. Rats were kept in individual metal cages with raised screen bottoms to prevent access to feces and were fed *ad lib* the diets

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† Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Hubbel, Mendel and Wakeman Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

§ Vitamin B_{12} Oral Grade Solids, Chas. Pfizer & Co., Brooklyn, N. Y.

‖ HY-DEE Powder, Standard Brands, Inc., New York City.

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¶ Similar to U.S.P. XII depletion diet except that commercial casein rather than extracted (vitamin A-free) casein was employed.

** MYVA-DRY Powder, Distillation Products, Inc., Rochester, N. Y.

TABLE I.
Effects of Low Environmental Temperature on the Rate of Depletion and Length of Survival of Immature Rats Fed a Vitamin A-free Diet (10 animals per group).

Initial body wt, g	Values end of depletion period		Avg survival after depletion*		Body wt after 50 days of feeding, g
	Avg age, days	Avg wt, g	Days	Range	
		Cold room series			
55.2	40.4	73.4	4.0 ± 0.5	(2–6)	—
54.6	—	—	—		136.4
		Room temperature series			
53.1	44.3	107.3	20.4 ± 1.4	(12–27)	—
52.8	—	—	—		233.1

* Including standard error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

listed above. Feeding was continued for 50 days or until death, whichever occurred sooner.

Results are summarized in Table I. Data were computed on the basis of the top 10 animals in each group in order to minimize variations in averages due to early deaths, infection and atypical responses on the part of individual rats. The end of the depletion period was arbitrarily set as the 5th day on which animals on a vit. A-free diet showed stationary or decreasing body weight. Findings indicate that rats fed a vit. A-free diet under cold room conditions differed from those fed a similar ration at room temperature in the following respects: (1) rats depleted more rapidly under cold room conditions, (2) body weight at time of depletion was less in the cold room than in room temperature series and (3) length of survival after depletion was significantly decreased in the cold room series. Gain in body weight was also reduced in animals fed the A+ diet under cold room conditions. These rats, however, did not develop xerophthalmia or other manifestations of vit. A deficiency as did animals on the vit. A-free diet. In accordance with previous findings(6) gangrene of the tail tip and edges of the ear was common in virtually all rats in the cold room series with amputations of the entire tail occurring in approximately 20% of the series. In general, however, these effects occurred with equal frequency on both diets employed.

Present findings indicate that prolonged exposure to cold increases the vit. A requirement of the rat. This is indicated by the fact that animals maintained on a vit. A-free diet depleted more rapidly under cold room than room temperature conditions and also by the shorter survival time after depletion of vit. A-deficient rats under conditions of low environmental temperature. A similar decrease in post-depletion survival time has been observed in vit. A-deficient rats following growth hormone administration(7). It is possible, however, that the decreased length of survival after depletion may reflect not a more rapid exhaustion of body stores of vit. A (as was suggested in the case of growth hormone) but rather failure of some physiological mechanism in the vit. A-depleted rat which is essential for resistance to cold.

Summary. Immature rats were fed purified rations deficient in vit. A under cold room (2°C) and room temperature (23°C) conditions. The following results were obtained: (1) rats depleted more rapidly under cold room conditions, (2) body weight at time of depletion was less in the cold room than room temperature series, and (3) length of survival after depletion was significantly decreased in the cold room series.

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