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Effects of Vitamin A Malnutriture on Resistance to Stress.* (19453)

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Considerable data are available indicating that resistance to stress may be significantly impaired in the nutritionally-deficient animal. Thus an impaired resistance to cold has been demonstrated in rats deficient in pyridoxine (1), riboflavin(2), and vit. A(3,4), and guinea pigs deficient in vit. C(5). An impaired resistance to anoxia has been reported in the riboflavin-deficient rat(6,7); and failure to excrete water and to withstand water intoxication has been observed both in riboflavin-deficient(8) and pyridoxine-deficient (9) rats. In the present communication data are presented on the effects of graded doses of vit. A on resistance to 1) low environmental temperature and 2) x-irradiation injury in the rat.

Procedure. The animals employed in the present experiment were male rats of the University of Southern California strain. The mother rats were maintained for at least 2 months previous to breeding and for 12 days after the birth of the litter on Sherman diet B(10) without addition of supplementary lettuce or meat. Litters were cut to 7 at 3 days of age, and on the 12th day mothers and litters were placed on a vit. A-low diet.† The young were weaned at 21-24 days of age and at a body weight of 36-50 g inclusive. The basal ration employed in the present experiment had the following composition: sucrose, 60%; casein,‡ 25%; salt mixture,§ 5%; cottonseed oil (Wesson), 10%; and the following synthetic vitamins per kg of diet: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 60 mg; ascorbic acid, 200 mg; biotin, 5 mg; folic acid, 10 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; vit. B_{12}, 150 μg; 2-methyl-naphthoquinone, 10 mg; and choline chloride, 2 g. To each kg of diet were also added 400 U.S.P. units of vit. D.‖ Each rat also received once weekly a supplement of 4.5 mg alpha-tocopherol acetate. Animals were kept in metal cages with raised screen bottoms to minimize access to feces and were fed *ad libitum.* Diets were made up weekly and stored under refrigeration. Rats were fed on alternate days. All food not consumed 48 hours after feeding was discarded to minimize oxidative changes in the diet.

Results. Exp. 1. Effects of graded doses of vit. A on survival time of rats under cold room and room temperature conditions. Rats averaging 43.4 g in weight were divided into 5 groups of 30 each and were fed the basal vit. A-free ration. They received in addition,

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† Similar to U.S.P. XIII depletion diet except that commercial casein rather than extracted (vit. A-free) casein was employed.

‡ Vitamin Test Casein, General Biochemicals, Chagrin Falls, O.

§ Hubbel, Mendel and Wakeman Salt Mixture, General Biochemicals, Chagrin Falls, O.

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

TABLE I. Effects of Graded Doses of Vit. A on Growth and Survival of Rats Under Cold and Room Temperature Conditions.

Avg daily supplement of vit. A, U.S.P. units	No. of animals	Initial body wt, g	Gain in body wt, 30 day period, g*	% surviving	Avg survival of decedents, days†
		Cold room series, $2 \pm 1.5°C$			
0	15	111.5	—	0	$1.9 \pm .6$
2.5	12	153.5	31	58	16.2 ± 2.7
5	12	156.4	20.8	92	$10 \pm .0$
10	12	154.3	24.9	100	—
50	12	163.7	34.1	100	—
		Room temperature series, 23°C			
0	15	105.1	—	0	19.3 ± 1.2
2.5	18	151.7	82.7	100	—
5	18	152.6	93.7	100	—
10	18	149.6	105.1	100	—
50	18	163.4	109.9	100	—

* Of surviving animals.

† Including stand. 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.

twice weekly by graduated syringe, oral supplements of vit. A¶ dissolved in 0.12 cc of cottonseed oil in amounts furnishing an average daily intake of 0, 2.5, 5, 10, and 50 U.S.P. units of vit. A for groups I to V, respectively. Rats in group I became depleted of vit. A (as judged by stationary or decreasing body weight for a period of 5 days) after an average of 23.4 days of feeding (range 21 to 27 days) and at an average body weight of 108.3 g. At the time of depletion half the animals were placed (1 animal per cage) in a walk-in refrigerator maintained at a temperature of $2° \pm 1.5°C$. The remaining half were continued at standard laboratory conditions (23°C). After 24 days of feeding the average body weight of rats in the remaining dietary groups was as follows: group II, 152.3 g; group III, 154.0 g; group IV, 151.3 g; and group V, 163.5 g. At this time animals in the above groups were divided into 2 series. Eighteen rats in each group were continued at standard laboratory conditions; the remaining 12 animals were placed in the walk-in refrigerator (1 animal per cage). No change was made in the diets fed. Feeding was continued *ad libitum* for an additional 30 days or until death, whichever occurred sooner. Results are summarized in Table I.

¶ Vit. A Palmitate (Synthetic), Hoffmann-La Roche, Nutley, N. J.

In agreement with earlier findings(3,4), exposure to a low environmental temperature significantly decreased the survival time of rats depleted of vit. A. At an environmental temperature of $2 \pm 1.5°C$, the average length of survival of rats depleted of vit. A was 1.9 days in contrast to an average survival time of 19.3 days for depleted rats at room temperature. An average daily intake of 2.5 U.S.P. units of vit. A was insufficient to provide maximal adjustment to cold since 42% of the rats in this series succumbed during an experimental period of 30 days. An average daily intake of 5 U.S.P. units of vit. A was sufficient, however, to permit an apparently optimal adjustment to cold. Under room temperature conditions all rats fed an average daily intake of 2.5 U.S.P. units of vit. A or more survived. Gain in body weight was correlated at room temperature conditions with the amount of vit. A administered, being significantly greater for rats fed the higher doses of vit. A than the lower. The differences between the various groups, however, were not marked; and with the exception of size the various groups were grossly indistinguishable. It is apparent from these findings that an average daily intake of 2.5 U.S.P. units of vit. A, although sufficient for good growth and survival under room temperature conditions, was inadequate for optimal adjustment to cold.

Exp. 2. Effects of graded doses of vit. A on mean arterial pressure of rats maintained under cold and room temperature conditions. At the termination of Exp. 1, 6 rats were selected at random from each of the surviving groups and the mean arterial pressure determined by the direct method.** The rats were anesthetized with ether and the abdominal aorta was exposed and punctured with a 24 gauge needle connected to a mercury manometer of 1 mm bore tubing. The puncture was made during light anesthesia and with the mercury column in the manometer raised to a level of 90-100 mm Hg. In the cold room series the average mean arterial pressure was 108.3, 95.4, 95.8, and 91.4 mm Hg, respectively, for rats fed an average daily intake of 2.5, 5, 10, and 50 U.S.P. units of vit. A. In the room temperature series the average mean arterial pressure of these groups was 101.5, 91.8, 95.4, and 98.2 mm Hg, respectively. The differences observed between rats fed the varying levels of vit. A were not statistically significant either under cold room or room temperature conditions; nor were the differences between the 2 environmental series on any of the rations employed. The latter findings are at variance with the report(11) that prolonged exposure to cold causes a significant increase in the systolic blood pressure of the rat. Gilson's determinations, however, were made with a tail plethysmograph on unanesthetized rats fed a natural food ration. In unpublished findings from this laboratory it was observed, in agreement with Gilson, that prolonged exposure to a low environmental temperature resulted in a significant increase in the systolic blood pressure of rats on a natural food ration, as determined in unanesthetized animals with a Sobin tail plethysmograph. When the mean arterial pressure was determined on these same rats, however, by the direct method outlined above, no increase in mean arterial pressure was observed over that of animals fed a similar ration but maintained under room temperature conditions. It would appear, therefore, either 1) that the hypertension caused by prolonged exposure to cold is abolished by ether anesthesia or 2) that the tail plethysmograph is not an accurate method for determining the systolic blood pressure of rats which had been exposed to a low environmental temperature for a prolonged period of time.

Exp. 3. Effects of graded doses of vit. A on survival of rats administered a single lethal dose of x-irradiation. At the termination of Exp. 1, 10 rats were selected at random from each of the surviving groups in the room temperature series and were administered a single dose of 750 r x-irradiation. Animals were continued on the basal ration plus their respective vit. A supplements for 30 days post-irradiation. The radiation factors were as follows: GE Model Maximar 250: 250 KV; 15 MA; 0.5 mm Cu and 1 mm Al filters plus a Cu parabolic filter;†† HVL, 2.15 mm Cu; target distance to top of box, 78 cm; and dose rate, 17.92 r per minute (measured in air). The animals to be irradiated were placed in a wooden box divided into 14 compartments 7 cm wide, 16 cm long and 10 cm deep (10 rats were irradiated at a time). The partitions and top were made of ⅛-inch cellulose acetate sheeting; and the top, one side and bottom of each compartment were perforated with holes for purposes of ventilation. The container was rotated slowly on an electrically-driven turntable to insure equivalent irradiation. The per cent survival and the average survival time of decedents in the various groups are summarized in Table II.

A significant difference was observed in the survival rate of rats fed varying doses of vit. A following a single exposure of 750 r

** We are indebted to Dr. Sheldon Rosenfeld, Dept. of Physiology, University of Southern California, for technical assistance in the blood pressure determinations.

†† A non-uniform filter which produces a flat isodose surface of x-ray intensity constructed by the method of Greenfield and Hand(12). We are indebted to Dr. Moses Greenfield and Miss Katherine Hand of the Atomic Energy Project, University of California at Los Angeles, for construction of the parabolic filter. The center of the filter had a thickness of 1.7 mm Cu; the edge, 0.5 mm Cu.

TABLE II. Effects of Graded Doses of Vit. A on Survival of Rats Administered a Single Lethal Dose of X-Irradiation. 10 rats in each series.

Avg daily supplement of vit. A, U.S.P. units	Initial body wt, g	Change in body wt, 30 days post-irradiation, g*	% surviving	Avg survival of decedents, days†
2.5	217.4	—	0	8.8 ± .8
5	276.3	–17.8	50	18.5 ± 3.8
10	279.3	–.4	50	14.4 ± 2.3
50	292.7	10.9	90	9 ± .0

* Of surviving animals. † Including stand. error of the mean.

x-irradiation. All of the rats receiving 2.5 U.S.P. units of vit. A daily succumbed, whereas, 50% of the rats fed 5 or 10 U.S.P. units, and 90% of those on 50 U.S.P. units of vit. A survived. During the first 4 days post-irradiation, rats in all groups lost approximately 20-25 g in body weight. Surviving animals fed the higher doses of vit. A gained back the weight they had initially lost. This did not occur, however, in rats fed an average daily intake of 5 U.S.P. units of vit. A. An unexpected finding was the occurrence of circular necrotic patches on the tail of irradiated rats fed an average daily intake of 5 or 10 U.S.P. units of vit. A (Fig. 1). These first became manifest during the second week post-irradiation and persisted for approximately 2 weeks in those animals which survived. Seventy percent of the rats fed an average daily intake of 5 U.S.P. units of vit. A and 90% of those receiving an average daily intake of 10 U.S.P. units of vit. A developed such patches. No such patches were observed in any of the rats receiving an average intake of 50 U.S.P. units of vit. A daily. Rats receiving an average daily intake of 2.5 U.S.P. units of vit. A succumbed following x-irradiation without showing such lesions.

Discussion. Results of the present experiment indicate that doses of vit. A sufficient to promote good but suboptimal growth under standard laboratory conditions are inadequate for optimal resistance to stress as caused by 1) prolonged exposure to a low environmental temperature or 2) a single exposure of 750 r x-irradiation. No data are available to indicate what factors were responsible for the impaired resistance of vit. A-deficient rats to the above stressor agents. Since food intake was reduced in the deficient rats, it is possible that the decreased resistance of these animals was due, at least in part, not to vit. A malnutriture *per se* but the attendant reduction in caloric intake. Further experiments are

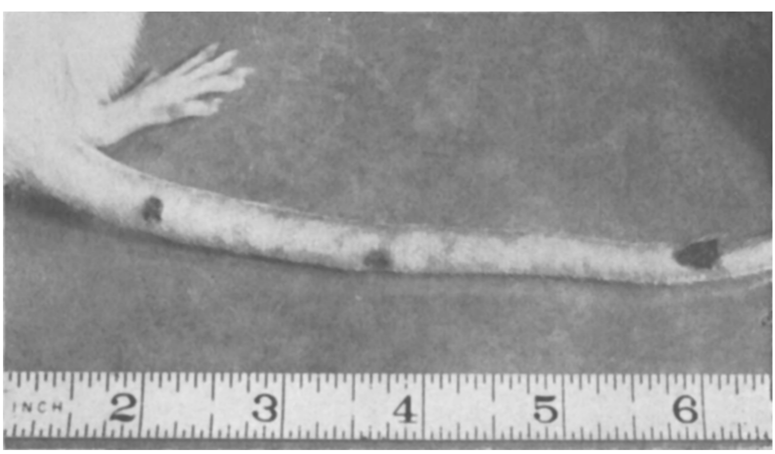

FIG. 1. Rat tail, 18 days post-irradiation, of animal fed a purified basal ration supplemented with average daily dose of 10 U.S.P. units of vit. A.

indicated to determine to what extent this factor may have contributed to the observed results.

Summary. Experiments were conducted on the effects of graded doses of vit. A on resistance to low environmental temperature and x-irradiation injury in the male rat. The average survival time of rats depleted of vit. A was 1.9 days at an environmental temperature of 2°C and 19.3 days under standard laboratory conditions (23°C). Adjustment to low environmental temperature (as judged by per cent surviving an experimental period of 30 days) was significantly increased in animals fed an average daily intake of 2.5 U.S.P. units of vit. A, but a minimal intake of approximately 5 U.S.P. units of vit. A daily was required for optimal adjustment to cold. A direct correlation was observed between the vit. A content of the diet and resistance to x-irradiation. All rats administered an average intake of 2.5 U.S.P. units of vit. A daily succumbed following a single exposure of 750 r x-irradiation; 90% of the rats receiving a daily intake of 50 U.S.P. units of vit. A and exposed to a similar dose of x-irradiation survived. An average daily intake of 10 U.S.P. units of vit. A was inadequate for optimal resistance to x-irradiation, although this level of vit. A was adequate under standard laboratory conditions for optimal growth.

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Reduction of Urinary Sodium and Potassium Produced by Hypophyseal Growth Hormone in Normal Female Rats.* (19454)

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Previous work from this laboratory(1) has shown that the administration of hypophyseal growth hormone (GH) to diabetic male rats results in the retention of both sodium and potassium even though the degree of glycosuria may be increased concurrently. It is the purpose of this paper to report that GH produces both sodium and potassium retention in normal female rats.

Methods. Three experiments are reported in this paper designated as Series I, II, and III. In each experiment 10 "plateaued" female rats of the Long-Evans strain were used. Five animals of each group were fed the regular stock diet† and 5 received the same stock diet plus an additional 600 mg of KCl

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† The stock diet consisted of ground whole wheat 67.5%, casein 15.0%, whole milk powder 10.0%, NaCl 0.75%, $CaCO_3$ 1.5%, hydrogenated vegetable oil, 5.25%. To each kg of diet were added 3.5 g Sardilene (fish oil concentrate containing 3000 USP units of vit. A and 400 chick units of vit. D per g).