Decreased Resistance of Riboflavin-Deficient Rats to Cold Stress.* (19445)

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Considerable data are available indicating that resistance to cold stress may be significantly impaired in the nutritionally-deficient animal. Thus an impaired resistance to cold has been demonstrated in rats deficient in vit. A(1,2) and pyridoxine(3) and guinea pigs deficient in vit. C(4). In the present communication data are presented indicating that riboflavin deficiency also impairs adjustment to low environmental temperature in the rat.

Procedure and results. Five experimental rations were employed in the present investigation: diets A, B, C, D, and E. Diet A was a riboflavin-free ration of 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; 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 4000 U.S.P. units of vit. A§ and 400 U.S.P. units of vit. D.‖ The vitamins were added in place of an equal amount of sucrose. Each rat also received once weekly a supplement of 4.5 mg alpha-tocopherol acetate. Diets B, C, D, and E were similar in composition to diet A, but contained in addition the following amounts of riboflavin per kg of diet: diet B, 0.5 mg; diet C, 1.5 mg; diet D, 10 mg; and diet E, 50 mg. One hundred and eight female rats of the Long-Evans strain were selected for the present experiment at 23 to 28 days of age and an average weight of 58.4 g. Animals were kept in metal cages with raised screen bottoms to prevent access to feces and were fed the above diets *ad libitum* (diet A was fed to 28 rats; diet B to 22; the remaining groups consisted of 20 rats each). Diets were made up weekly and stored under refrigeration when not in use. Animals were fed on alternate days. All food not consumed 48 hours after feeding was discarded. These measures were employed to minimize oxidative changes in the diet. After 3 weeks of feeding the following average body weights were obtained for rats in the various dietary groups: diet A, 71.2 g; diet B, 115.8 g; diet C, 118 g; diet D, 143.2 g; and diet E, 144.5 g. At this time 25 of the 28 rats on diet A were depleted of riboflavin as judged by stationary or decreasing body weight for a period of 7 days; all rats on diets B, C, D, and E were gaining weight. Animals in the various dietary groups were then divided into 2 series. Ten rats in each group were continued at standard laboratory conditions (23°C); the remaining animals in each group were placed in a walk-in refrigerator maintained at a temperature of 2 ± 1.5°C. No change was made in the diets fed. Feeding was continued *ad libitum* for an additional 8 weeks or until death, whichever occurred sooner. Results are summarized in Table I.

Findings indicate that rats depleted of riboflavin failed to survive under conditions of low environmental temperature. One hundred per cent of the rats fed diet A died under cold room conditions with an average survival time of 7.6 days (range 2 hours to 17 days). Under room temperature conditions, however, 60% of the rats fed a similar ration were still

* This paper reports research undertaken in cooperation with the quartermaster Food and Container Institute for the Armed Forces, and has been assigned No. 371 in the series of papers approved for publication. The views or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views of indorsement of the Department of the Army.

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

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

§ Myva-Dry Powder, Distillation Products, Rochester, N. Y.

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

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

Dietary groups	Riboflavin in ration, mg/kg of diet	No. of animals	Initial body wt, g	Change in body wt after 8 wk exp., g	% surviving*	Avg survival time of decedents, days
			Cold room series			
A	0	16	71.6	—	0	7.6
B	.5	12	116.6	−5 (3)	25	22
C	1.5	10	120.9	35 (8)	80	22
D	10	10	146.6	33.9 (10)	100	
E	50	10	144.1	32.4 (10)	100	
			Room temp. series			
A	0	10	70.5	−13.8 (6)	60	43
B	.5	10	114.9	7.5 (10)	100	
C	1.5	10	115.1	57.2 (10)	100	
D	10	10	139.7	57.3 (10)	100	
E	50	10	141.8	66.2	100	

Values in parentheses indicate number of animals surviving on which averages are based.
* Experimental period—56 days.

alive 8 weeks after depletion. A dietary intake of 0.5 mg riboflavin per kg of ration was insufficient to maintain survival under conditions of low environmental temperature. Seventy-five per cent of the rats fed this diet succumbed under cold room conditions with an average survival time of 22 days (range 15 to 33 days). At room temperature conditions, however, this intake of riboflavin was sufficient to keep all rats alive and to promote a slight increment in body weight. Higher levels of riboflavin (1.5, 10 and 50 mg per kg of ration) permitted growth and survival both under cold room and room temperature conditions.

Discussion. It is well established that requirements for adrenal cortical hormones are markedly increased under conditions of low environmental temperature(5-7) and that adrenalectomized or hypophysectomized rats fail to survive following prolonged exposure to cold. Adjustment to cold is also impaired by thyroidectomy(8) or administration of thiouracil(9). Resistance to cold may be restored in adrenalectomized rats by administration of adrenal cortical hormone(s) and in thyroidectomized or thiouracil-fed rats by injection of thyroxin. Present findings indicate that resistance to low environmental temperature (as judged by length of survival) was significantly impaired in rats fed rations containing 0.5 mg of riboflavin per kg of diet or less. Since considerable impairment of adrenal cortical function may occur in the riboflavin-deficient rat(10), further studies are indicated to determine to what extent adrenal cortical (and possibly pituitary and thyroid) dysfunction may have contributed to the decreased resistance of riboflavin-deficient rats noted above. Preliminary findings indicate that neither cortisone acetate¶ (administered at a level of 2.5 mg per day intraperitoneally) nor thyroxin (administered at a level of 30 γ per day intraperitoneally) were effective in prolonging the survival of riboflavin-deficient rats under conditions of low environmental temperature.

Results of the present experiment indicate that a state of nutriture which may be adequate under standard laboratory conditions may be inadequate under conditions of cold stress. Thus riboflavin when administered at a level of 0.5 mg per kg of diet was insufficient to maintain life and body weight under conditions of low environmental temperature, whereas this level was sufficient to keep all rats alive and to promote a slight increment in body weight under room temperature conditions. It is possible, since food intake was reduced in the riboflavin-deficient rats, that the impaired adjustment of riboflavin-deficient rats to cold stress was due, at least in part, not to riboflavin malnutriture *per se* but the attendant reduction in caloric intake. Further experiments are indicated to deter-

¶ The cortisone acetate was kindly provided by Dr. R. A. Peterman of Merck and Co., Rahway, N. J.

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

Summary. Experiments were conducted on the effects of graded doses of riboflavin on the growth and survival of rats maintained under cold room and room temperature conditions. Weanling rats were fed for 3 weeks on diets containing 0, 0.5, 1.5, 10, and 50 mg of riboflavin per kg of diet. After 3 weeks of feeding (at which time rats on the riboflavin-free diet had plateaued in weight) animals were divided into 2 groups. One group was placed in a cold room maintained at 2°C; the other was maintained at room temperature (23°C). Adjustment to cold (as measured by length of survival) was significantly impaired in rats fed diets containing 0.5 mg of riboflavin per kg of diet or less.

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Received December 27, 1951. P.S.E.B.M., 1952, v79.

Presence of Bacteria in the Spleen of Pteroylglutamic Acid Depleted Rats. (19446)

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Gross lesions, infarct like in nature, were reported by Asenjo(1) to develop in the spleens of a considerable number of pteroylglutamic acid (PGA) depleted rats receiving a basal PGA deficient diet containing either succinylsulfathiazole (SST) or x-methyl-folic acid as inhibitor. Later Philips and Thiersch (2) studied the action of 4 amino-pteroylglutamic acid on rats and observed, in 5% of the animals receiving this compound by chronic administration, the appearance of intestinal lesions through which an ascending Salmonella infection took place. As a result of this bacterial invasion enlargement of the mesenteric lymph nodes, abscess formation, multiple fibrinoid necrosis in liver, spleen, kidney, and lung, bronchitis and bronchopneumonia occurred.

The above findings have led us to search for bacteria in the gross splenic lesions obtained in PGA depleted rats receiving SST as inhibitor.

Experimental. Thirteen 21-day-old Wistar albino rats from our colony were kept in cages with raised wire floors. The basal purified diet free of PGA, fed *ad libitum,* had the following composition per kg: sucrose, 599 g; vitamin-free casein, 180 g; salt mixture, 40 g; cellu flour, 40 g; hydrogenated vegetable oil, 100 g; corn oil, 20 g (containing 52,000 I.U. vit. A, 13,000 I.U. vit. D, and 32 mg α-tocopherol); succinylsulfathiazole, 20 g; choline chloride, 1 g; inositol, 8 mg; nicotinic acid, 40 mg; calcium pantothenate, 44 mg; thiamine hydrochloride, 8 mg; riboflavin, 16 mg; pyridoxine hydrochloride, 8 mg; p-aminobenzoic acid, 4 mg; biotin,† 0.5 mg; 2-methyl-1,4-naphthoquinone, 10 mg. The depletion period ranged from 3 to 4 weeks. By the end of the fourth week the majority of the animals

* Project sponsored by the Agric. Exp. Station of the University of Puerto Rico.

† Biotin supplied by Hoffmann-La Roche, courtesy of Dr. E. Sevringhaus.