highest. In our studies with rats, the organ of maximum uptake following intravenous injection of acetate buffered $Cr^{51}Cl_3$ was also the bone. With $Cr^{51}Cl_3$ in rats and sheep the organs of maximum concentration were the liver and lung, respectively. The high uptake by the lung of the sheep may have resulted from increased phagocytosis of particulate material by the lungs of these animals due to a lung infection which had been prevalent in the flock.

Summary. The physiological behavior of trivalent and hexavalent Cr^{51} has been observed over a 45-day period in rats as affected by chemical form and route of administration. When intravenously administered as sodium chromite, practically 100% of the dose was picked up by the organs of the reticuloendothelial system. Chromic chloride resulted in a pickup by the liver of about 55% of the dose. When $Cr^{51}Cl_3$ was added to isotonic acetate and citrate buffers, less than 5% of the dose reached the liver and most of the activity was excreted in the urine. The use of sodium chromate solutions resulted in about 25% of the dose reaching the liver. However, the activity left this organ rapidly in contradistinction to the other forms studied. Oral, intraperitoneal and intratracheal administration of $Cr^{51}Cl_3$ were also studied. None of the forms of Cr^{51} used were found to cross the placenta of rats ranging from 15 to 20 days in gestation. In addition, data are presented from 2 sheep intravenously dosed with $Cr^{51}Cl_3$. The above physiological observations are correlated with the colloidal behavior, complex formation, protein binding, and red cell binding, and are in general agreement with interpretations of filter paper electrophoretic studies of the dosing solutions.

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Decreased Resistance of Vitamin B_{12}-Deficient Rats to Cold Stress.* (20730)

BENJAMIN H. ERSHOFF.

From the Emory W. Thurston Laboratories, Los Angeles, Calif.

Considerable data are available indicating that requirements for a number of nutrients are increased and resistance to cold stress is decreased in the nutritionally-deficient animal. In the present communication data are presented on 1) the effects of prolonged exposure to cold on the vit. B_{12} requirement of the rat and 2) the effects of vit. B_{12} deficiency on resistance to cold stress in the rat.

Procedure. The basal ration employed in the present experiment consisted of expeller-processed soybean flour,† 66%; sucrose,

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† Soy Flour Lo-Fat, A. E. Staley Mfg. Co., Decatur, Ill.

24.4%; salt mixture,‡ 4%; cottonseed oil (Wesson), 3%; wheat germ oil (VioBin), 2%; and dl-methionine, 0.6%. To each kg of the above diet were added the following synthetic vitamins: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 60 mg; biotin, 2 mg; 2-methylnaphthoquinone, 5 mg; folic acid, 10 mg; ascorbic acid, 200 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; vit. B_{12}, 100 μg; and choline chloride, 2 g. To each kg of diet were also added 8000 U.S.P. units of vit. A§ and 800 U.S.P. units of vit. D.‖ The vitamins were added in place of an equal amount of sucrose. Tests were conducted 1) with rats fed the basal ration and 2) with animals fed a similar diet with vit. B_{12} omitted.

Results. Exp. 1. Seventy female rats of the Long-Evans strain were selected at 25 to 28 days of age and at a body weight of 52 to 60 g and were fed the diets indicated above for 12 weeks or until death, whichever occurred sooner. Tests were conducted 1) with animals kept continuously in a large walk-in refrigerator at a temperature of 2 $\pm$ 1.5°C and 2) under standard laboratory conditions at an average temperature of approximately 23 $\pm$ 2°C. Rats were kept in metal cages with raised screen bottoms to prevent access to feces and were fed the above diets *ad lib.* (20 rats per group in the cold room series; and 12 and 18 rats per group on the basal ration and deficient diet respectively in the room temperature series). 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.

Findings indicate that under room temperature conditions all rats tested survived the 12-week feeding period. The average gain in body weight of rats on the basal ration during this period was 140.8 $\pm$ 3.8 g,¶ and the average weight increment of rats fed the vit. B_{12}-deficient diet was 126.9 $\pm$ 5.3 g.¶ The difference in weight increment between these 2 groups was not statistically significant.** In the cold room series gain in body weight was retarded in all rats. Fifteen out of 20 (75%) of the rats fed the basal ration survived the 12-week experimental period with a weight increment of 93.5 $\pm$ 4.9 g;¶ 14 out of 20 (70%) fed the vit. B_{12}-deficient diet also survived with a weight increment of 69.8 $\pm$ 3.0 g.¶ Gain in body weight of rats fed the basal ration was statistically significantly greater than that of rats fed the vit. B_{12}-deficient diet. Grossly no significant differences were observed between rats in the various groups. Since the weight increment of rats fed the vit. B_{12}-containing ration was significantly greater under cold room conditions than that of rats fed a similar diet with vit. B_{12} omitted, whereas no significant difference in weight increment was observed in rats fed these diets under room temperature conditions, it would appear that requirements for vit. B_{12} were increased by exposure to low environmental temperature under conditions of the present experiment.

Exp. 2. Following the 12-week feeding period in Exp. 1, animals on both diets in the room temperature series were bred to normal males. Nine litters were cast by rats on the vit. B_{12}-deficient diet of which 3 were weaned at 21 days of age at an average weight of 32.4 g per rat. Six of the litters and 2 of the mothers succumbed during the lactation

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

§ Crystalets, Chas. Pfizer and Co., New York, N. Y.

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

¶ 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.

** The failure of rats fed the vit. B_{12}-deficient diet to exhibit growth retardation under room temperature conditions was probably due to pre-test storage of vit. B_{12} during the period of pregnancy and lactation(12). That this diet was actually deficient in vit. B_{12} was demonstrated by the growth retardation and post-weaning deaths of the young born to mothers on the depletion diet in *exp. 2* and the absence of these disorders in rats fed a similar diet supplemented with crystalline vit. B_{12}.

period. Twelve litters were cast by rats on the basal ration of which 6 were weaned at 21 days of age at an average weight of 35.2 g per rat. The remaining 6 litters died during the lactation period. Following weaning the young in each group were continued on the same diet for an additional 30 days or until death, whichever occurred sooner. In agreement with earlier findings(1) a number of the young on the vit. B_{12}-deficient diet (5 out of 18) died within 3 weeks after weaning. Six male rats and 7 female rats, however, survived the 30-day post-weaning period with an average increment in body weight of 38.9 $\pm$ 4.4 g¶ and 32.3 $\pm$ 3.4 g,¶ respectively. In contrast all the young on the basal ration (17 males and 20 females) survived the 30-day post-weaning period with an average gain in body weight of 98.7 $\pm$ 6.5 g,¶ and 75.4 $\pm$ 2.6 g¶ respectively. At this time the surviving rats in the vit. B_{12}-deficient group and 10 male and 10 female rats of the group fed the basal ration were placed in a cold room maintained at a temperature of 2 $\pm$ 1.5°C. No change was made in the diets fed. Feeding was continued for an additional 21 days or until death, whichever occurred sooner. All rats fed the vit. B_{12}-deficient diet died during the first 96 hours of cold exposure with over half the animals succumbing within the first 18 hours. In contrast, all rats fed the basal ration survived the 3-week period of cold exposure.

Discussion. Available data indicate that requirements for a number of nutrients are increased under conditions of low environmental temperature. This is particularly true for some of the water-soluble vitamins. An increased requirement for thiamine(2,3), riboflavin(4), pyridoxine(5), and ascorbic acid (6) has been demonstrated following prolonged exposure to cold. In addition resistance to cold stress was significantly impaired in rats deficient in pyridoxine(7), riboflavin (8), pantothenic acid(9), and vit. A(10,11), or fed restricted amounts of a complete ration(9). It is becoming increasingly apparent that the inability of deficient rats to withstand sudden exposure to cold is due to an impaired adjustment to this type of stressor agent and not to an increased nutritional requirement and the consequent accentuation of the deficiency state. Present findings indicate that requirements for vit. B_{12} are similarly increased under conditions of low environmental temperature and that rats deficient in vit. B_{12} also have a decreased resistance to cold.

Summary. 1. Prolonged exposure to cold significantly increased the vit. B_{12} requirement of the rat. 2. Resistance to cold stress (as measured by length of survival under conditions of low environmental temperature) was significantly impaired in the vit. B_{12}-deficient rat.

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