TABLE I. Cortisone and ACTH on Various Types of Induced Pain.

Type of pain stimulus	Subject	Hormone	Before hormone (range)	During hormone (range of change)
I Cutaneous pain*	5 patients	4 cortisone, 1 ACTH	1+ to 3+	0 to −1+ **
" " †	5 "	4 " 1 "	7+ to 8+	−1+ to +1+ **
II Pain threshold‡	5 "	Cortisone	157 to 232 mc	−10 to +8 mc ††
III Visceral pain	6 "	4 cortisone, 2 ACTH	40 to 75 §	−20 to 0 § ††
	1 "	Morphine sulfate	50 §	+100 § ††
	4 "	3 cortisone, 1 ACTH	1.7 to 3 ‖	−.7 to +.4 ‖ ††
	1 "	Morphine sulfate	1.7 ‖	+1 ‖ ††
IV Pain from radiant heat	5 rats	Cortisone	6.2 to 8.6¶	0 to +1.4 ¶ ††

Stand. dev. were calculated for all hormone experiments and in no instance was the change found statistically significant.

* Intradermal NaCl. † Intradermal distilled water. ‡ As measured by Wolff-Hardy-Goodell apparatus using dorsum of hand. § Volume of air in balloon in cc required to produce a sensation. ‖ Pressure of air in balloon in cm Hg required to produce a sensation. ¶ Seconds. ** Decrease denotes analgesic effect. †† Increase denotes analgesic effect.

ACTH is probably due to an hormonally-induced alteration in the response of the host to the inflammatory reaction(14,15) or to a parallel decrease in the inflammatory reaction not necessarily affecting the etiologic agent(16).

Summary. A systematic evaluation of cutaneous, dental and visceral pain before and during the administration of either cortisone or ACTH demonstrated that these agents had no analgesic effect at therapeutic dose levels. The marked alteration in the host reaction to inflammation during treatment with cortisone or ACTH probably is the principal factor responsible for the alleviation of pain.

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Decreased Resistance of Pyridoxine-Deficient Rats to Cold Exposure.* (19081)

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It is well established that the pituitary-adrenal system is activated under conditions of stress(1,2), and that requirements for ACTH(3) and adrenal cortical hormone(s) (4,5) are increased during adjustment to cold. Hypophysectomy (with the consequent reduction in ACTH secretion) and adrenalectomy (with the resultant decrease in adrenal cortical hormones) both impair adjustments to cold(4,6). A deficiency of

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thyroid hormone caused by thyroidectomy(7) or the administration of antithyroid drugs(8) also impairs survival under conditions of low environmental temperature. Various dietary factors including ascorbic acid(9), vitamin A(10) and riboflavin(11) are also essential for optimal adjustment to cold. In the present communication data are presented on the effects of pyridoxine deficiency on resistance to cold stress in the rat.

Procedure and results. The basal 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 diet 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, 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. Each rat also received 3 times weekly a vit A-D concentrate§ containing 50 U.S.P. units of vit A and 5 U.S.P. units of vit D, and once weekly 4.5 mg of alpha-tocopherol acetate. Tests were conducted(1) with rats fed the basal ration and (2) with animals fed a similar diet with the pyridoxine hydrochloride omitted.

Exp 1. Fifty-two female rats of the Long-Evans strain were selected at 25 to 28 days of age and an average weight of 56.0 g and fed the diets indicated above for 100 days, or until death. 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. Rats were kept in metal cages with raised screen bottoms to prevent access to feces and were fed the above diets *ad lib* (16 rats per group in the cold room series; 10 animals per group 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 an experimental period of 100 days. This occurred on both the basal and pyridoxine-free diet. Gain in body weight was significantly greater on the control than the deficient diet, but animals in both groups gained weight continuously throughout the experimental period. No dermatitis occurred in any of the rats fed the pyridoxine deficient diet and with the exception of body weight no significant differences were observed grossly between the 2 groups. After 100 days of feeding the average gain in body weight of rats on the basal ration was 176.0 ± 4.2 g,‖ and the average weight increment of rats fed the pyridoxine deficient diet was 99.2 ± 5.1 g.‖ In the cold room series growth was retarded in all rats. Thirteen out of 16 (81.3%) of the

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

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

§ Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vit. A and 80,000 U.S.P. units of vit. D per g.

‖ 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.

rats fed the basal ration survived the experimental period of 100 days with a weight increment of 122.6 ± 6.5 g;‖ 12 out of 16 rats (75.0%) fed the pyridoxine-deficient diet also survived with a growth increment of 64.4 ± 2.8 g.‖ As in the case of the room temperature series animals continued to gain weight continuously throughout the experimental period and no dermatitis or other manifestations of pyridoxine deficiency (with the exception of growth retardation) was observed grossly in any of the rats fed the pyridoxine-deficient diet.

Exp. 2. Fifty-two female rats of the Long-Evans strain 23 to 25 days old and averaging 46.2 g in weight were divided into 2 groups of 26 rats each and fed *ad lib* under standard laboratory conditions for a period of 80 days the basal ration and pyridoxine-deficient diets indicated above. After 80 days of feeding the average weight increment of rats fed the basal ration was 172.8 ± 5.4 g‖ and of animals fed the pyridoxine-deficient diet, 97.7 ± 6.3 g.‖ At this time each group was divided into 2 parts. Sixteen rats in each group were placed in a cold room maintained at a temperature of 2 ± 1.5°C; the remaining 10 animals in each group were continued at standard laboratory conditions (23° C). No change was made in the diets fed. Feeding was continued for an additional 20 days or until death, whichever occurred sooner. In the room temperature series all rats survived the additional 20 days of feeding with a weight increment of 17.9 g for animals on the basal ration and 6.3 g for rats fed the pyridoxine-deficient diet. In the cold room series, however, 11 out of the 16 rats fed the pyridoxine-deficient diet (68.8%) died within the 20 day period (average survival time 12.6 days); all rats fed the basal ration survived. The average loss in body weight during cold exposure was 12.4 g for rats on the basal ration and 33.5 g for surviving animals on the pyridoxine-deficient diet.

Discussion. Present findings indicate that rats raised to maturity on a pyridoxine-deficient diet readily succumb following cold exposure but continue to survive on a similar diet under room temperature conditions. The cause of death in such animals is apparently not due to an accentuation of the pyridoxine deficiency as a result of cold exposure but rather to a failure in the mechanism of adjustment to cold. This is indicated by the fact that no significant reduction occurred in the survival of rats fed a pyridoxine-deficient diet when such animals were placed in the cold prior to the time they were pyridoxine-deficient. It would appear, therefore, that pyridoxine is essential for optimal adjustment to cold but that dietary sources of this vitamin are dispensable for survival (at least in the rat) once adjustment to cold has occurred.

In contrast to earlier investigations under room temperature conditions in which rats fed a diet deficient in pyridoxine plateaued in body weight, developed dermatitis and died, animals fed a pyridoxine-deficient ration under conditions of the present experiment gained weight continuously and showed no gross manifestations of pyridoxine deficiency with the exception of retardation in growth. The cause for this discrepancy is not readily apparent. It is not unlikely, however, that the higher B vitamin and fat content in the present experiment stimulated a greater intestinal synthesis of pyridoxine or promoted a better utilization of any available pyridoxine-active nutrients.

Available data do not indicate what factors were responsible for the impaired adjustment of pyridoxine-deficient rats to a low environmental temperature. In preliminary studies(12) no significant difference was observed between rats fed a pyridoxine-deficient diet and animals fed a similar ration supplemented with pyridoxine hydrochloride in the leukocyte picture after epinephrine administration. Both groups showed a reversal in the differential count, an increase in total leukocytes, a marked increase in polymorphonuclear leucocytes and a significant reduction in lymphocytes and eosinophils 3 hours after epinephrine administration. These responses are indicative of an activated pituitary-adrenal system and are similar to those which occur in the normal animal after ACTH or

12. Parrott, F. D., Jr., and Ershoff, B. H., unpublished data.

cortisone administration or in response to non-specific stress(13,14). Twenty-four hours after the injection of epinephrine the blood picture of both pyridoxine-deficient and control rats had returned to pre-injection levels in respect to all cellular constituents with the exception of the eosinophils which were still reduced by over 50%. These findings indicate that pyridoxine deficiency does not impair the ability of the pituitary-adrenal system to respond to acute stress. The activation of the adrenals which occurs in response to epinephrine administration, however, is based primarily if not entirely on the release of preformed ACTH. It is possible that the pyridoxine-deficient rat may have sufficient amounts of this hormone stored in its pituitary to effect a normal response to acute stress but that such rats may have an impaired ability to produce the increased amounts of ACTH required in chronic stress. The role of pyridoxine in protein metabolism suggests the possibility that animals deficient in this vitamin may have an impaired ability to synthesize proteins including adrenocorticotropin, thyrotropin, and others. In view of the increased requirement for thyrotropin(15) and adrenocorticotropin(3) during prolonged exposure to cold, the failure of pyridoxine-deficient rats to adjust to low environmental temperature may result not from pyridoxine deficiency *per se* but rather from a deficient secretion of pituitary hormones. Further experiments are indicated to determine the effects of thyroid, ACTH and cortisone administration on the survival of pyridoxine-deficient rats under conditions of low environmental temperature.

Summary. Immature rats were fed a purified ration containing all the known B vitamins in synthetic form and a similar diet with pyridoxine omitted. Tests were conducted in which rats fed the above diets were exposed to cold ($2 \pm 1.5°C$) from the first day of feeding or after an experimental period of 80 days. 68.8% of the pyridoxine-deficient rats in the latter group succumbed during a 20 day period of cold exposure in contrast to a 0% mortality for animals fed a similar diet supplemented with pyridoxine hydrochloride. In the group exposed to cold from the first day of feeding mortality was 25.0% and 18.7% respectively for rats fed the pyridoxine-deficient and control ration for a period of 100 days.

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Preparation of Specific Complement-Fixing Antigen with Lansing Poliomyelitis Virus.* (19082)

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The complement fixation (CF) technic has been successfully applied to the study of many of the human viral encephalitides with a notable exception of poliomyelitis. Recently Casals and Olitsky(1) described the preparation and use of a specific CF antigen prepared from MEF_1 virus adapted to new-born mice. Pollard(2) described the preparation of a CF antigen from Lansing infected cotton rat (CR) tissues by precipitation of the virus with methanol and use of the eluate as anti-

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