The Lability of Thiamine in Certain Purified Rations.* (18933)

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Hankes and Elvehjem(1) described the development of a nervous condition in rats upon feeding a low protein, niacin-free, ration supplemented with DL-phenylalanine and DL-methionine for a period of 10-12 weeks. The symptoms included an arched back, oscillation of the head, loss of equilibrium, and convulsions which were followed by death in 36 to 50 hours. It was recognized that the symptoms resembled a thiamine deficiency but the following conditions, adequate supply of thiamine in the ration (.2 mg %), delayed development of the symptoms, and complete prevention of symptoms when 18% of casein was used or when niacin or tryptophan were supplied from the beginning of the experiment, suggested a separate and distinct syndrome. When these studies were continued, considerable variation was encountered in the production of the symptoms and finally injections of thiamine were tried. The injection of a rather large single dose (80 μg) alleviated the convulsions and the continued administration of the vitamin restored normal growth in the animals.

We wish to report in this paper the rapid destruction of thiamine in some of the rations used and the remarkable stabilizing effect of succinylsulfathiazole. Any specific effect of phenylalanine and methionine in such rations cannot be determined until the level of thiamine is more accurately controlled.

Experimental. Forty-five male weanling rats of the Sprague-Dawley strain were placed in separate screen-bottomed cages and divided into 9 groups of 5 animals each. All were placed on a thiamine-free basal ration for 1 week. The different groups were then fed the rations listed in Fig. 1. The basal ration consisted of sucrose 79.8%, corn oil 5%, casein† 9%, salts IV(2) 4% and DL-methionine 0.2%. A niacin-free vitamin mix‡ diluted with sucrose was added at a 2% level. In series 2 the thiamine was omitted from the vitamin mix. Thus the animals in series 1 received the same level of thiamine (0.2 mg/100 g ration) and in the same manner as those in the previous studies. The rations for groups 2, 4, 6 and 8 were also supplemented with 1.5% succinylsulfathiazole in order to minimize any synthesis of thiamine in the tract. Kratzing and Slater(3) have shown that this drug does not contribute to the thiamine economy of the rat.

All the animals in series 2 received the thiamine by injection. The thiamine solution was prepared weekly and contained 10 μg per ml. Initially each animal received 10 μg daily but this was reduced to 8 μg at the end of the second week and to 5 μg at the end of the fifth week. This level was then maintained until the end of the experiment.

The thiamine content of the rations and the livers from the rats in series 2 was determined by the thiochrome method described by John-

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1. Hankes, L. V., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 349.

† Casein extracted 3 times with hot ethanol.

2. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, v138, 459.

‡ Vitamins were added as a dry mix and provided per 100 g of ration; thiamine-HCl 0.2 mg, riboflavin 0.3 mg, pyridoxine 0.25 g, calcium DL-pantothenate 2 mg, choline chloride 100 mg, inositol 10 mg, biotin 0.01 mg, and folic acid 0.02 mg. In series 2 the thiamine-HCl was omitted. Periodic analysis of the mix showed that the thiamine destruction was negligible over a 10-12 week period. Halibut liver oil fortified with vitamin K and vitamin E was administered by dropper once weekly.

3. Kratzing, C. C., and Slater, E. C., *Biochem. J.*, 1950, v47, 24.

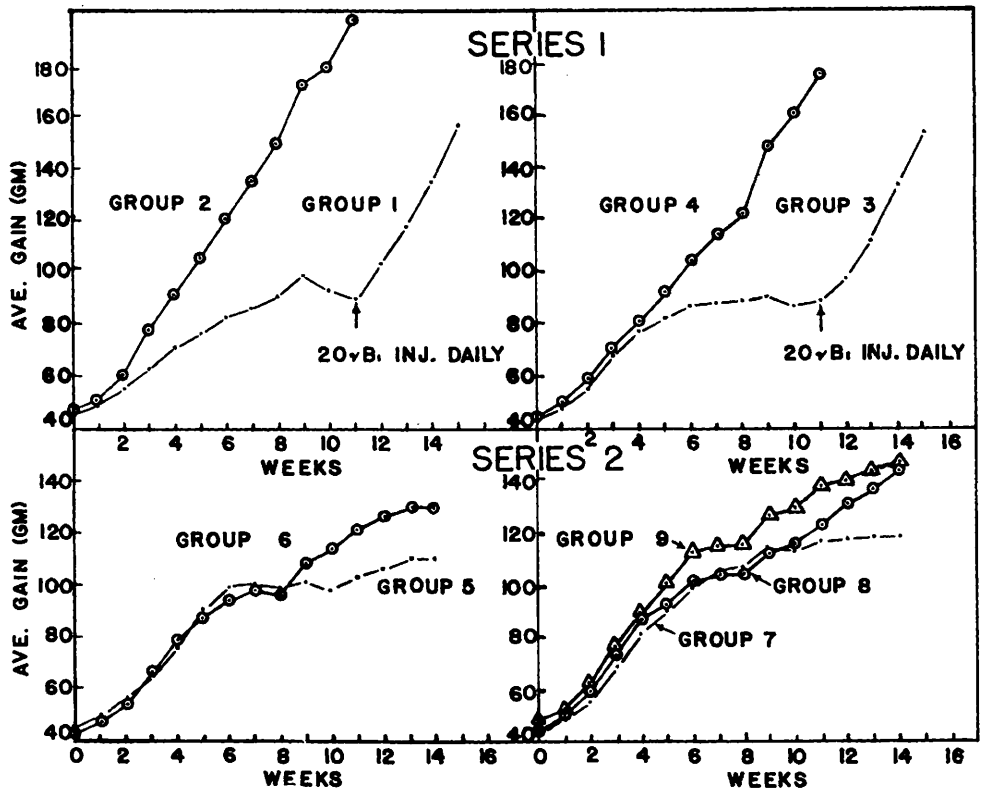

FIG. 1. Growth response of rats fed a niacin-free 9% casein basal ration under the following conditions: Series 1—thiamine incorporated directly into the ration. Group 1, basal ration; group 2, basal ration + 1.5% sulfasuxidine; group 3, basal ration + 0.2% DL-phenylalanine; group 4, basal ration + 0.2% DL-phenylalanine + 1.5% sulfasuxidine. Series 2—5 μg thiamine injected intraperitoneally daily. Group 5, basal ration; group 6, basal ration + 1.5% sulfasuxidine; group 7, basal ration + 0.2% DL-phenylalanine; group 8, basal ration + 0.2% DL-phenylalanine + 1.5% sulfasuxidine; group 9, basal ration + 0.2% DL-phenylalanine + 1.5 mg % niacin.

son(4) and by Gyorgy(5). Extraction of the samples was carried out in most cases in 0.1 N H_2SO_4, with steaming for 30 minutes. In addition to the steaming, the livers were incubated with pepsin and takadiastase for 18 hours after adjusting the pH to 4.5 with sodium acetate.

Results and discussion. The growth data are shown in Fig. 1. The rats in group 1, control, and group 3, with added DL-phenylalanine, grew slowly until the eighth or ninth week, at which time the gains in both groups ceased. By the eleventh week all the animals in both groups had developed convulsions. When these animals were given a single injection of 80 μg of thiamine, all were cured of the symptoms except one animal in group 3 which died upon injection. Continued intraperitoneal injections of 20 μg of thiamine daily restored normal growth and appearance of the animals in both groups. In contrast to the poor growth exhibited by the animals in groups 1 and 3, those in groups 2 and 4 grew remarkably well. Food consumption records showed no significant differences between the groups during the first 3 weeks with an average consumption of 5-6 g of ration per rat per day. By the eighth week the animals in groups 1 and 3 were eating about 4.5 g and those in groups 3 and 4, almost 10 g per day. The only difference in the rations was the presence of sulfa drug. The effect could not

4. Johnson, C. B., *Methods of Vitamin Determination,* Burgess Publishing Co., 1948, p52.

5. Gyorgy, P., *Vitamin Methods,* v1, Academic Press Inc., Publ. 1950, p94.

TABLE I. Results of Thiamine Analysis of Rations Used in Series 1* and Livers from the Rats in Series 2.

Supplement	Series 1 Group No.	Series 1 B_1 content of ration after 6 days, $\mu g/g$	Series 2 Group No.	Series 2 B_1 content of liver. Avg of 3 animals, $\mu g/g$ liver
None	1	.52	5	1.32 ± .03†
1.5% sulfasuxidine	2	1.9	6	1.20 ± .09
0.2% DL-phenylalanine	3	.56	7	1.35 ± .19
" " + 1.5% sulfasuxidine	4	1.7	8	1.30 ± .13
0.2% DL-phenylalanine + 1.5 mg % niacin			9	1.21 ± .14

* Determinations made on rations 6 and 8 showed no fluorescence, so the sulfasuxidine was not contributing to the values of rations 2 and 4.

† Standard error $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

be due solely to alterations in the intestinal flora which might allow more thiamine to be available to the host. If this had been true, groups 6 and 8 of series 2, receiving identical rations except for a daily injection of a limited amount of thiamine, should have shown a similar growth response. The increased growth observed in groups 6 and 8 over that observed in groups 5 and 6 may have been due to an increased availability of tryptophan resulting from the effect of the sulfa drug since the growth response was quite similar to that of group 9 which received 1.5% of niacin in the ration. The rate of growth of all the rats in series 2 was undoubtedly limited by the lower supply of thiamine. These animals consumed only 5-6 g of ration per day. Further evidence that the sulfa drugs did not alter the availability of thiamine is shown in Table I. The thiamine levels in the livers of the rats from the different groups are very similar.

In series 1 the rats on the niacin-free basal control ration developed convulsive symptoms as frequently as those receiving added DL-phenylalanine. In series 2 no increased tendency toward the convulsions was exhibited by the rats in group 7 which received the phenylalanine. Thus, it appears that phenylalanine does not directly increase the demand for thiamine.

The reason for the superior growth response of the animals receiving sulfasuxidine became evident when the ration was analyzed for thiamine. Examples of typical analyses are shown in Table I. The original rations contained 2 μg of thiamine per g ration. While 70-75% was destroyed in the non-sulfasuxidine mixture, nearly all of the vitamin was recovered from the rations containing the drug. Thus the animals of groups 2 and 4 received from 15-20 μg of thiamine per day, while animals of groups 1 and 3 received less than 5 μg per day. Actually the amount must have been considerably less than 5 μg since those in series 2 received 5 μg and showed no signs of convulsions. The protection afforded by the sulfa drug does not seem to be one of bacterial inhibition in the mixed ration for as much as 50% destruction can be shown in a matter of hours after preparing the ration. This rapid destruction is also shown by a lack of growth response when a freshly mixed ration is fed. Very little depletion of the vitamin occurs during storage of the vitamin mix over long periods. In an effort to determine whether the method of extraction was exaggerating the destructive effect, samples were extracted with cold water or else sulfasuxidine was added just before the acid extraction. However, analyses made in this way within a short time after preparing a fresh mix indicated up to 50% destruction which increased to as much as 80% within 2 weeks.

The exact cause of this vitamin destruction is not known, but present information indicates that the salts IV used in the ration provides the destructive agent or agents. Preliminary work has shown that potassium iodide

in relation to the copper sulfate and ferric citrate may provide the destructive agent. De Ritter and Rubin(6) showed that ferrous iron would destroy thiamine under conditions of acid extraction, but that this destruction could be avoided by the addition of cystine to the extraction solution. It is of some interest to note that when L-cystine replaced the DL-methionine as the sulfur amino acid supplement, no convulsions were observed and growth was considerably improved. However, cystine added to the extraction solution or to the ration does not improve the recovery of the vitamin.

The data presented in this paper are based on a limited number of animals but similar results have been obtained with many other groups of animals. In all cases when a reduced growth response has been obtained a definite loss of thiamine in the ration as revealed by chemical analyses has been demonstrated. While more work is needed to establish the exact mechanism of the destruction and the protective action of the sulfa drug, it seemed advisable to bring these effects to the attention of other workers. Since the lability of thiamine has been encountered in rations containing only 9% casein and no added niacin or tryptophan, one might conclude that protein and tryptophan or niacin may have some protective effect. However, it is more likely that a deficiency of tryptophan or niacin may reduce the food intake sufficiently to precipitate a thiamine deficiency when a significant portion of that added to the ration is destroyed. These relationships may be studied further when the level of the thiamine in the ration can be accurately controlled.

Summary. The "nervous syndrome" condition produced in rats after 10 weeks or longer on a low protein, niacin-free regimen has been shown to respond to thiamine supplementation. Complete protection from the convulsions was affected when sulfasuxidine was added to the ration. Analyses of the rations for thiamine showed that as much as 70% of the vitamin was destroyed in the non-sulfasuxidine ration, while very little destruction occurred in the ration containing the drug. The addition of 0.2% DL-phenylalanine did not increase the thiamine requirement.

6. De Ritter, E., and Rubin, S. H., *Ind. Eng. Chem.*, Anal. Ed., 1947, v19, 243.

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Use of Cortisone in Treatment of Experimental Frostbite. (18934)

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It has been reported that cortisone and ACTH therapy has been beneficial in the treatment of severe burns(1,2). Since frostbite resembles burn injuries in some respects, cortisone has been used in the treatment of frostbite casualties in the Korean conflict. It has been demonstrated that the adrenal glands become enlarged in rabbits following local cold injury, and in severe frostbite pathologic changes have been observed in the cortices(3). However, as far as we know, no clinical or experimental evidence has been presented to support the use of cortisone in local cold injuries. These experiments were performed to determine the effect of this drug on the extent of frostbite necrosis.

Materials and methods. The experiments were performed on 65 male albino rabbits of more than 2,500 g body weight. Two degrees of local cold injury were induced, one by exposure of a hind leg to an alcohol bath at −12°C for 30 minutes for the determination of muscle necrosis, and the other at −15°C for 30 minutes for the determination of skin as well as muscle necrosis. We have shown in

1. Crasweller, P. O., Farmer, A. W., Franks, W. R., and McLachlin, A. D., *Brit. M. J.*, 1950, v2, 977.
2. Whitelaw, M. J., and Woodman, T. W., Letter to the Editor, *J. Clin. Endocrinol.*, 1950, v10, 1171.
3. Hoelscher, B., to be published.