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Growth of Rats on Synthetic Diets Containing Limiting Amounts of Thiamine.*

J. M. McIntire, L. M. Henderson, B. S. Schweigert, and C. A. Elvehjem.
From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Richardson and Hogan[1] reported that on a synthetic diet the 8 known vitamins were the only essential vitamins necessary for a rat to attain normal weight and maturity. Henderson *et al.*[2] and Unna *et al.*[3] also reported successful growth and reproduction on similar rations but found that when these diets were supplemented with whole liver or liver extracts greater growth responses were obtained than when the synthetic rations were fed alone.

Since optimum growth rarely results in assay experiments it has undoubtedly occurred to many workers that these simplified diets might be used for the determination of the individual vitamins in natural products. This procedure would greatly simplify some of the biological methods now in use. However one important question must be answered, namely, will a unit amount of a vitamin give the same response on these diets when fed as a free vitamin as when it is supplied in natural materials which carry many other factors?

We have attempted to answer this question in the case of thiamine and some of the results are presented in this paper.

Experimental. Several yeast and liver preparations were studied to determine which would produce the greatest growth responses when added to the synthetic ration with and without thiamine supplementation. Thirty-two male rats were divided into 2 groups. One group received ration 227 which consisted of casein, 18%; sucrose, 75%; salts IV, 4%; corn oil, 3%; pyridoxine, 200 γ; riboflavin, 300 γ; nicotinic acid, 250γ; pantothenic acid, 2 mg; and choline, 100 mg per 100 g of ration; the other group received ration 227 plus 150 γ of thiamine per 100 g of ration. All rats received one drop of halibut liver oil weekly. Yeast and liver preparations were fed to 2 rats of each group and were added to the ration at the expense of the sugar. The average weekly gains of these rats over a 5-week experimental period are shown in Table I.

The rats which received ration 227 plus 2% sulphited liver extract[4] (ration 228) plus 150 γ of thiamine per 100 g of ration grew better, 35.7 g per week, than did the rats on any of the other rations. The rats on ration 228 became polyneuritic at the same time as the controls on ration 227. This eliminated the possibility that the liver extract furnished thiamine to the ration.

To determine the differences in the rate of growth of animals on these rations with various levels of thiamine 8 groups of 3 rats each were placed on ration 227 and 228 supplemented with 0, 60, 80, 100, and 150 γ of thiamine per 100 g of ration. In every case the growth of the rats on ration 228 was superior to the growth on ration 227, Table II. The greatest difference in growth on the 2 rations appeared in rats receiving 80 γ of thiamine per 100 g of ration.

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3 Unna, K., Richards, G. V., and Sampson, W. L., *J. Nutrition*, 1941, **22**, 553.

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TABLE I.
Growth of Male Rats Receiving Ration 227 Supplemented with Various Liver and Yeast Preparations.

Supplement	Growth in g per week	
	0.0 γ thiamine	150 γ thiamine
None	polyneuritis 4 weeks	28.9
2% 1:20 sulphited liver extr.	" " "	35.7
5% sulphited yeast	" " "	32.1
2% butanol extr. of 1:20 liver extr.	" " "	30.6
0.5% 1:20 liver extr.	" " "	30.8
1% liver fraction B	" " "	25.0
5% autoclaved yeast	" " "	29.0
2% solubilized liver fraction L	" " "	26.0

The sulphited liver extract was autoclaved for 3 hours at pH 5.7 to determine the stability of the active factors present. The preparation was fed at a level of 2% to a group of 3 rats receiving ration 227 plus 80 γ of thiamine per 100 g of ration. Since none of the activity was lost it was thought that p-aminobenzoic acid and inositol might be the active compounds. An experiment was devised to study the effect of these substances: 4 groups of 3 male rats each were fed the following rations, 227 plus 80 γ of thiamine; 227 plus 80 γ of thiamine and 30 mg of p-aminobenzoic acid; 227 plus 80 γ of thiamine and 100 mg of inositol; and 227 plus 80 γ of thiamine, 30 mg of p-aminobenzoic acid, and 100 mg of inositol per 100 g of ration. The results of these experiments are shown in Table III.

TABLE II.
Growth of Male Rats Receiving Rations 227 and 228 Supplemented with Various Levels of Thiamine.

γ of thiamine per 100 g of ration	Growth in g per week	
	Ration 227	Ration 228
0.0	polyneuritis 4 wks	polyneuritis 4 wks
60.0	4.0 g	11.0 g
80.0	7.9	20.0
100.0	18.0	27.0
150.0	27.0	33.0

TABLE III.
Growth of Rats Receiving Rations 227 Supplemented with Inositol, p-Aminobenzoic Acid and Liver Extract.

Supplement per 100 g of ration	No. rats	Growth g/wk
80 γ thiamine	9	12.8
2% autoclaved sulphited 1:20 liver extr. + 80 γ thiamine	3	20.0
30 mg p-aminobenzoic acid + 80 γ thiamine	3	12.7
100 mg inositol + 80γ thiamine	3	14.2
30 mg p-aminobenzoic acid, 100 mg inositol and 80 γ thiamine	9	16.3

Rats which received the p-aminobenzoic acid or inositol respectively failed to show growth responses over that obtained on ration 227. However when the two compounds were fed together a definite growth response was obtained. Considerable variation was observed in these studies but in 3 individual experiments rats receiving both p-aminobenzoic acid and inositol showed a definite growth increase over those receiving ration 227.

Discussion. Considerably better growth was obtained when sulphited liver extract was added to the synthetic ration even when suboptimal levels of thiamine were added. The greatest growth differences were observed when 80 γ of thiamine were fed per 100 g of ration. It is thus impossible to use a synthetic ration for assay purposes since the natural materials to be studied may cause a growth stimulation similar to that obtained with the liver preparation.

A comparison of the growth curves of rats receiving ration 227 and 228 to which 0, 80, and 150 γ of thiamine were added per 100 g of ration is interesting (Fig. 1). The growth of rats on the two rations receiving 150 γ of thiamine was very similar, especially after 6 or 7 weeks, although the rats receiving the liver extract grew faster during the first 5 weeks on experiment. The rats on ration 228 which received 80 γ of thiamine grew considerably faster than those on ration 227, although

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animals on both rations plateaued at the same time, approximately 4 weeks. The animals on the basal rations became polyneuritic at the same time. These facts eliminate the possibility of a thiamine-sparing action.

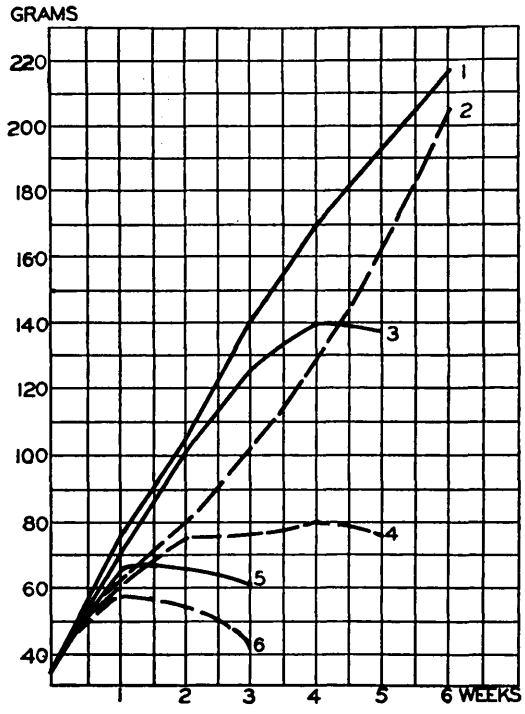

FIG. 1.

Growth curves of rats receiving: 1, 228 plus 150 γ thiamine; 2, 227 plus 150 γ thiamine; 3, 228 plus 80 γ of thiamine; 4, 227 plus 80 γ of thiamine; 5, 228 basal; and 6, 227 basal.

The increased growth produced by the liver preparation may be explained on the basis of one of two mechanisms. Either the extract furnished a growth-stimulating substance which has a greater effect when suboptimal levels of thiamine are fed, or it furnished a condition for an intestinal flora which synthesizes growth-stimulating factors that are produced to some extent when the rat is receiving a normal diet. This seems possible since similar effects have been noted on suboptimal pantothenic acid rations for growth,[3] and for protection against grey hair.[5]

The p-aminobenzoic acid and inositol probably exert the same effect that the liver extract does. Martin[6] has suggested that p-aminobenzoic acid alters the intestinal flora in achromotrichia to affect the synthesis of "folic acid." Pavcek[7] reported that inositol showed a growth-promoting activity when fed to pyridoxine-deficient animals. Inositol has recently been shown to play a role in lactation of the rat,[8] and Engel[9] states that inositol is a necessary constituent in highly purified diets for the rat.

Ration 228 has been used as a basal ration for the determination of thiamine in crude materials. Both curative[10] and growth technics have been used. Results obtained by the growth method and the thiochrome assay method show good correlation but studies involving a much larger number of rats are necessary before a more exact comparison of the two methods can be made.

Summary. Superior growth was obtained when synthetic diets containing various levels of thiamine were supplemented with sulphited liver extract. The addition of p-aminobenzoic acid and inositol to the synthetic ration also increased the rate of growth of rats receiving suboptimal levels of thiamine.

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9 Engel, R. W., *J. Nutrition*, 1942, **24**, 175.

10 Waisman, H. A., and Henderson, L. M., unpublished data.