

Thiamine and Anti-Microbial Defenses¹

P. LEONARD KNIGHT, University of Notre Dame

Introduction

Studies on thiamine metabolism have been carried out in our laboratory for several years; e.g., thiamine synthesis by bacteria of the digestive tract of the rat using radiosulfate as a precursor, the nature of the thiamine in the cecum, and thiamine-sparing action of penicillin. Recently, it has come to our attention, that thiamine may play an integral part in the antimicrobial defenses of the rat. This conclusion is based upon the results of studies of thiamine content of the liver and heart, of the regional blood flow to the liver and of the transketolase activity of the liver. In all experiments the comparison between germfree and conventional animals permitted an evaluation of the effect of the presence of a viable microbial flora.

Both germfree and conventional rats, age 21 days to 3 months, were used in these studies. Earlier work in our laboratory had shown that the heart of the germfree rat was 15-25% smaller by weight than the heart of its conventional counterpart (2). This fact in turn indicated that there might be a difference in circulation between the two groups. Cardiac output and regional blood flow were therefore determined as follows: The former was measured via the indirect Fick principle, using Sapirstein's adaptation (4) of the indicator method of Hamilton et al. (3). Rb⁸⁶Cl was used as the test substance. Blood collections from cannulated carotids were made at the rate of 55/30 secs., each amounting to 0.03 ml. Blood flow to organs was determined after the method of Sapirstein again using Rb⁸⁶Cl as the test substance. Animals were sacrificed 30 secs. after administration of the isotope. This isotope maintains a stable level in all organs (except the brain) between 10 and 60 secs. post injection and thus the fractional uptake of the indicator by various organs is equal to their blood flow fraction of the cardiac output.

All animals were sacrificed by decapitation. Livers and hearts were removed and analyzed immediately or frozen for later use. Thiamine was determined by a modification of the thiochrome method as outlined by Wostmann and Knight (5). Analysis of transketolase activity was made after the method of Brin (1).

Results and Discussion

As has been mentioned previously, the heart of the adult germ-free rat is 15-25% smaller by weight than the heart of its conventional counterpart when the animals are on a complete thiamine diet (2), yet

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the amount of thiamine dihydrochloride/gram tissue (Table 1) is approximately the same in both groups.

TABLE 1

Thiamine in Hearts and Livers and Regional Blood Flow to Livers of Adult Germfree and Conventional Rats. S.D.M. Values Given.

	Thiamine 2HCl $\mu\text{gm/gm}$ Heart	Thiamine 2HCl $\mu\text{gm/gm}$ Liver	Regional Flow ml/min./gm liver
Germfree	6.7 (12) ± 0.1	6.8 (21) ± 0.1	0.27 (5) ± 0.04
Conventional	6.7 (12) ± 0.1	9.1 (18) ± 0.3	0.53 (4) ± 0.04

() Number of samples.

Liver data, however, show the reverse of that found for the hearts. Thus the livers of adult germfree and conventional rats are approximately the same weight (3500 mg%), but data in Table 1 shows that the amount of thiamine dihydrochloride/gm liver tissue in the germfree animal is 6.8 μgm while the amount in the conventional rat harboring a normal flora is 9 μgm . Furthermore, the regional blood flow to the liver of the conventional animal—0.53 ml/min./gm—is much greater than the flow to the liver of the germfree—0.27 ml/min./gm (6).

Data in Table 2 indicate that the levels of thiamine in the hearts of growing germfree and conventional animals (age 6-7 weeks) on diets containing various, but suboptimal amounts of thiamine, were again comparable. The livers of conventional animals however, generally contained more thiamine/gm tissue than livers of germfree animals when the amount of thiamine in the diet was the same. At the 8.8 μgm thiamine/gm diet level (a more than optimal amount for normal growth),

TABLE 2

Thiamine in Livers of Growing Germfree and Conventional Rats Fed Increasing Amounts of Thiamine in the Diet. Age: 6-7 Weeks.

Thiamine in Diet $\mu\text{gm/gm}$	Thiamine in $\mu\text{gm/gm}$ Tissue			
	Heart		Liver	
	Germfree	Conventional	Germfree	Conventional
0.4	0.4	0.5 ns
0.6	2.1	2.0 ns	0.5	1.0 hs
0.75	2.1	1.9 ns	0.6	1.0 ns
1.1	3.1	2.6 ns	1.0	1.2 s
1.5	3.0	3.0 ns	1.5	2.0 s
8.8	3.7	8.7 hs

ns—non-significant.

s —significant at 5% level.

hs—significant at 1% level.

the amount of thiamine/gm liver tissue in the conventional animal was more than double that found in germfree.

At this point the question poses itself: Is this difference in liver thiamine levels a bacterial production effect—more flora-synthesized thiamine available to the conventional animal or does the liver of the germfree animal require less thiamine because of the absence of a bacterial load effecting the clearance and detoxification mechanisms? The fact that even with suboptimal intake only the germfree liver shows a constantly lower thiamine level seems to indicate that lower requirement rather than lower intake is at the basis of this difference. This is confirmed by the observation that on complete diets, where the contribution of flora-synthesized thiamine to the host was proven to be negligible (5), the same difference exists.

A greater regional blood flow to the liver of conventional animals indicates that more O_2 is needed for the biological oxidation processes, presumably resulting in more chemical energy via catabolism to cover the energy requirements of the liver for clearance and detoxification of materials of bacterial origin. The importance of thiamine as thiamine pyrophosphate in these biological oxidations cannot be over-emphasized. It is known that thiamine is a component of at least 25 enzyme systems. Thiamine pyrophosphate (TPP) for example acts as a coenzyme in the removal of CO_2 from pyruvic acid at the end of the Embden-Meyerhof scheme of glucose metabolism. It is also a necessary component of several enzymes employed in the Krebs cycle and plays a significant role as a transketolase in the direct oxidative pathway of glucose. Analysis of liver transketolase by J. Green using ribose-5-phosphate as a substrate has shown no significant difference in organ content of germfree and

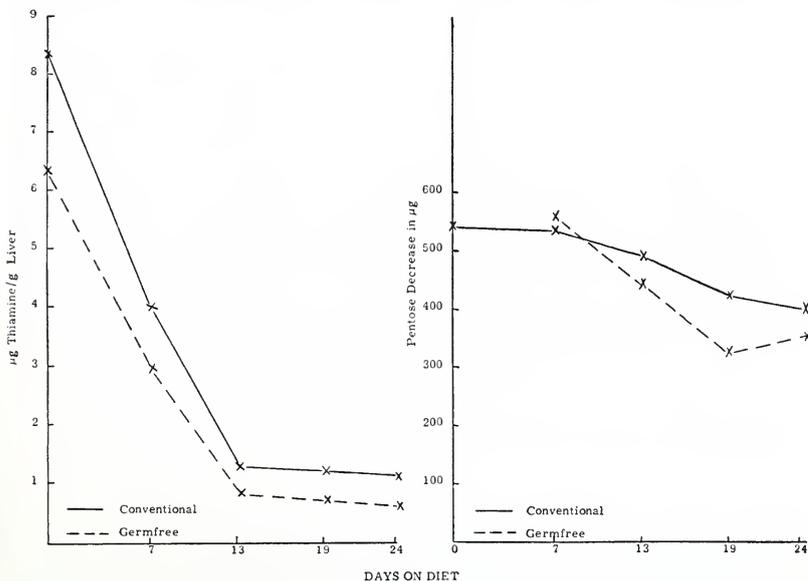


Figure 1

conventional rats. Data in Fig. 1 (a comparison of thiamine content and transketolase activity of the liver in germfree and conventional rats on a thiamine deficient diet) show that the thiamine levels after six days have dropped considerably in both germfree and conventional, while in conventionals the transketolase activity has not changed significantly during that time. Incomplete data at this time seems to indicate that transketolase activity in the liver of germfree rats after six days on the diet follows essentially the same pattern as that of the conventional. Only severe thiamine depletion of the liver causes the transketolase activity to decrease.

Assuming the lower thiamine level of the liver of the germfree rat is an expression of a reduced need for one or more thiamine pyrophosphate containing enzymes, it appears that transketolase is not the limiting enzyme in the energy production for the clearance and detoxification. The lower energy requirement of the germfree liver, expressing itself in lower thiamine levels and less regional blood flow to the liver than is seen in conventionals presumably manifests itself in the reduction of one or more of the other thiamine containing enzyme systems than that of transketolase. The data do express, however, the importance of sufficient thiamine uptake to maintain adequate enzyme levels, which will meet the requirement of the liver to maintain optimal functional capacity.

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Fig. 1. Comparison of thiamine content and transketolase activity of the liver in germfree and conventional rats on a thiamine deficient diet.