BIOCHEMICAL CHANGES IN THIAMINE DEFICIENCIES

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Thiamine deficiency has been associated with a triad of symptoms: Anorexia and weight loss; cardiomegally and bradycardia; and neuromuscular disturbances (ataxia and convulsions). Although much work has been done, the biochemical bases of these symptoms are still not entirely known.

Two antagonists of thiamine, oxythiamine (OTh) and pyrithiamine (PTh), have been used extensively to produce experimental thiamine deficiencies in animals. They are of particular interest since OTh produces most of the other symptoms but never the neurological symptoms, while PTh produces primarily the neurological symptoms. We have made extensive studies of the effects of these antagonists and of thiamine deprivation on various biochemical parameters in rats, and have attempted to correlate these changes with the appearance and severity of the various symptoms. Typical growth curves for these three types of deficiency are shown in Fig. 1.

Anorexia is most marked and appears earlier in OTh-treated rats, next in thiamine-deficient, and least in PTh-treated rats (1,2). In the latter, failure to eat and weight loss actually don't appear until neurological involvement (ataxia) is evident. As shown in Figs. 2 and 3, the appearance and severity of the anorexia correlated best with decreased activity of transketolase in the intestinal mucosa. Since OTh has been shown not to enter the brain and not to produce any neurological symptoms, it would suggest that the effect is probably not on the appetite center in the brain, unless it is an indirect effect of changed metabolite levels, e.g., the increased blood pyruvate level.

Various aspects of heart function and metabolism were studied in rats in which thiamine deficiency symptoms were produced by thiamine deprivation, and treatment with the antagonists OTh or PTh. The results are shown

![Fig. 1. Typical weight gain curves.](image-url)
Fig. 2. Mucosal pyruvate dehydrogenase activity of rat small intestine. ●—●, thiamine controls; ○—○, thiamine-deficient; △—△, OTh-treated; □—□, PTh-treated. † shows time of appearance of anorexia. The center well of the reaction vessel contained the following in μmoles: Phosphate buffer, pH 7.6, 37.4; versene, 1.0; MgSO₄, 10.0; ATP, 3.0; NAD⁺, 0.5; Na-fumarate, 2.2; sucrose, 25; and pyruvate, 10.0 containing 7.3 × 10⁻² μC (162,000 dpm) of 1-¹⁴C-pyruvate. The outer chamber contained 0.3 ml 0.1 M Hyamine 10X. The reaction was started by injecting 4.5 μmoles of K₃Fe(CN)₆ into the center well through the rubber stopper with syringe and needle. Total volume = 0.85 ml, t = 37°C, time = 30 min. Reaction stopped with 0.5 ml 1.0 N HCl and the ¹⁴CO₂ collected in Hyamine and measured in a scintillation counter. ⊕ denotes standard error.

Fig. 3. Mucosal transketolase activity in rat small intestine. ●—●, thiamine control; ○—○, thiamine-deprived; △—△, OTh-treated; □—□, PTh-treated. † denotes time of appearance of anorexia. Reaction mixture: Glycylglycine buffer, pH 7.3, 50 μmoles, MgSO₄, 6 μmoles, NADH₂, 0.2 μmole; glycerophosphate dehydrogenase-triosephosphate isomerase mixture, 50 mg; homogenate, 0.1 ml; H₂O, 1.4 ml. Reaction started by 0.1 ml pentose phosphate equilibrium mixture and A₃₄₀ read at 5-min intervals. ⊕ denotes standard error. (Figures 1 to 3 were reprinted by permission of Journal of Nutrition.)
Fig. 4. The effect of progressive thiamine deprivation on heart wt. (○—○) expressed as mg/100 g body wt.; creatine phosphate (▲—▲); heart rate (■—■); pyruvate dehydrogenase (□—□); and α-ketoglutarate dehydrogenase (△—△) activity. Each point represents the mean of 5–10 rats. The data are expressed as percentage of values obtained in normal control animals killed at the same time.

Fig. 5. The effect of progressive oxythiamine treatment on heart wt. (○—○) expressed as mg/100 g body wt.; creatine phosphate (▲—▲); heart rate (■—■); pyruvate dehydrogenase (□—□); and 2-ketoglutarate dehydrogenase (△—△) activity. Each point represents the mean of 5–10 rats. The data are expressed as percentage of values obtained in normal control animals killed at the same time.

Fig. 6. The effect of progressive pyrithiamine treatment on heart wt. (○—○) expressed as mg/100 g body wt.; creatine phosphate (▲—▲); heart rate (■—■); pyruvate dehydrogenase (□—□); and 2-ketoglutarate dehydrogenase (△—△) activity. Each point represents the mean of 5–10 rats. The data are expressed as percentage of values obtained in normal control animals killed at the same time.

(Figures 4 to 6 were reprinted from Sutherland et al. (3).)

in Figs. 4, 5 and 6 (3). The appearance and degree of severity of the bradycardia and cardiac enlargement correlated most closely with the decreases in pyruvate and α-ketoglutarate dehydrogenase activities in the heart. These cardiac changes didn’t seem to be related to an energy deficit since the creatine phosphate level did not decrease or in fact increased in the terminal stages of deficiency. Heart perfusion studies indicated that the bradycardia was not the result of the high pyruvate and lactate levels associated with thiamine deficien-
Bradydardia was found to persist in the isolated perfused hearts from deficient rats. After making appropriate corrections for inanition itself, a residual true cardiomegally and EEG irregularities were confirmed in rats in all three types of deficiency (3).

Studies on the effects of the three types of thiamine deficiency on the operation of the TCA cycle and the γ-aminobutyrate (GABA) shunt were made in rats (see Fig. 7). As shown in Figs. 8 and 9, all three types of deficiency showed a decrease in glutamate and increase in α-ketoglutarate (αKg) levels in both blood and brain along with a significant

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**Fig. 7.** The TCA cycle with the GABA shunt.

**Fig. 8.** Blood glutamate and α-ketoglutarate levels in control (□); thiamine-deficient (□); oxythiamine-treated (■); and pyrithiamine-treated (■) rats.

**Fig. 9.** Brain γ-aminobutyrate (GABA), glutamate and α-ketoglutarate levels in control and deficient rats (designated as in Fig. 8).

**Fig. 10.** Recovery of 14C in various TCA and GABA shunt intermediates from brain 30 min after the intracerebral injection of 2-14C-α-ketoglutarate in control rats and rats treated as in Fig. 8. □, control; □, deficient; ■, OTh; ■, PTh.
decrease in the brain GABA level (5). Brain glutamate dehydrogenase activity was not affected. Thirty minutes after the intracerebral injection of 2-14C-αKg, the 14C recovered in various TCA intermediates from the brain of rats with the three types of deficiency was determined. As shown in Fig. 10 the 14C recovered in brain αKg and glutamine was greater, while that in aspartate and glutamate was less in all three types of deficiency as compared to pair-fed control rats. The 14C in GABA was increased in PTh-treated rats but was not affected in OTh-treated or thiamine-deprived rats. The 14C in succinate was decreased in thiamine-deprived and PTh-treated, but was unaffected in OTh-treated rats. These results suggest that changes in the level or production of TCA or GABA shunt intermediates probably plays no direct role is the cause of the neurological symptoms.

Fig. 11. Changing relationship of brain wt. to body wt. with age and wt. of rats. ○, normal rats; ●, thiamine-deprived rats; △, oxythiamine-treated rats; □, pyrithiamine-treated rats. Each point represents the mean from 2 to 6 rats.

Fig. 12. Thiamine derivatives in rat brain after 25 days on treatment. □, control; ●, thiamine-deprived; Δ, OTh-treated; ●, PTh-treated; ○, brain PTh level in PTh-treated rats. A=total thiamine, B=ThDP+ThTP.

Fig. 13. Changes in levels of thiamine derivatives in rat brain with days on experimental treatment. ○—○, control; ●—●, thiamine-deficient; △—△, OTh-treated; ○—○, PTh-treated; ●—●, brain PTh level in PTh-treated rats. A=total thiamine, B=ThDP+ThTP.
Fig. 14. Concentrations of thiamine derivatives in brain with different levels of thiamine intake. 

![Graph showing concentrations of thiamine derivatives](image)

For a large series of rats in several experiments, the brain weights were plotted as percentage of body weight against body weight (see Fig. 11) [4]. This demonstrates that rats on experimental deficiencies which had attained a higher weight and then subsequently lost weight, had brain weights which were identical with rats which had attained the same body weight by uninterrupted growth. Thus, thiamine-deficient and antagonist-treated rats had brain weight/body weight ratios which were the same as either normal fed or pair-fed rats of the same body weight.

The three types of thiamine deficiency were produced in rats and the levels of total thiamine, ThDP+ThTP and hydroxyethyl thiamine (HET) determined in the brains of animals sacrificed at intervals. Figure 12 presents the values after 25 days on the treatment, i.e., terminal levels. PTh caused a marked depletion of all thiamine derivatives, thiamine deficiency much less marked, and OTh treatment caused little or no drop in brain levels. The time course of depletion of these thiamine derivatives is shown in Fig. 13 A and B. The rate of decrease of both total thiamine and ThDP+ThTP of the brain is very rapid in PTh-treated rats and correlates well with the increase in the brain PTh level. The rate of depletion is slower and the final levels attained only half as low with thiamine deprivation as with PTh treatment. The initial drop with the control and OTh-treated rats reflected the change from the purina chow diet to the lower intake on the experimental diet (i.e., 10 µg Th/100 g body weight/day, injected subcutaneously). In view of this difference in tissue levels with 10 µg/100 g/day, which had been shown to be entirely adequate for growth [9] and the levels in purine chow fed rats a study was made of the effects of thiamine intake on brain levels of thiamine derivatives. The results shown in Fig. 14, show that both total and phosphate ester forms of thiamine increased rapidly with intake up to 200 µg/100 g
body weight/day. Intake levels higher than this inhibited retention to some extent. HET levels were not as responsive to intake as were the other forms. Thus, though 10 μg/day intake is sufficient for normal growth, the tissue stores, even brain stores, are depleted at this level.

Studies were made to determine whether the branched-chain α-ketoacid dehydrogenase complexes in rat liver mitochondria (i.e., KIVDH, KICDH, and KMVDH) were subject to phosphorylation-dephosphorylation with the resulting inactivation-activation by the protein kinase-phosphatase system as reported for purified pyruvate dehydrogenase complex (PyDH) by Linn et al. (6) and others (7, 8). Dehydrogenase activities were determined by measuring the 14CO₂ liberated from 1-14C-α-ketoacid substrates by a method worked out in this laboratory (Gubler, unpublished). As shown in Fig. 15, in this crude mitochondrial system, not only was PyDH ac-

![Fig. 16](image_url) Changes in activity of αKgDH and PyDH of liver mitochondria at 10 mM Mg²⁺ (●—●) and 10 mM Mg²⁺ (○—○) with age of the rat.

![Fig. 17](image_url) Changes in activity of KICDH and KIVDH of liver mitochondria at 10 mM Mg²⁺ (●—●) and 10 mM Mg²⁺ (○—○) with age of the rat.
tivated under conditions (high \( \text{Mg}^{2+} \) concentration) which favored dephosphorylation by activation of the phosphatase, but \( \alpha \text{KgDH} \), \( \text{KIVDH} \), \( \text{KICDH} \), and \( \text{KMVDH} \) were also activated under these conditions.

The activities of \( \text{PyDH} \), \( \text{KgDH} \), \( \text{KIVDH} \), and \( \text{KICDH} \) in rat liver mitochondria were determined at intervals from age 21 through 64 days under conditions (10 mM and 100 mM \( \text{Mg}^{2+} \) concentrations) which favored inactive and active forms, respectively. As shown in Figs. 16 and 17, there was a marked increase in total activity and in the difference between inactivated and activated levels with all four substrates between 30 and 40 days of age. This interesting phenomenon is being further investigated. A study of the activity at various \( \text{Mg}^{2+} \) concentrations demonstrated that the optimum levels for phosphorylation and activation for all five dehydrogenases were 10 mM and 100 mM, respectively. These are the levels of \( \text{Mg}^{2+} \) which have been customarily used with \( \text{PyDH} \) studies. Earlier studies with \( \text{PyDH} \) have used 0.10 mM concentrations of ATP for activation-inactivation studies. A study was made in which the ATP concentration was varied from 0.005 to 6.0 mM at each of the two levels of \( \text{Mg}^{2+} \) (i.e., 10 mM and 100 mM). The results show that optimum activation of \( \text{PyDH} \), \( \alpha \text{KgDH} \), \( \text{KICDH} \), and \( \text{KMVDH} \) occurred at 0.50 mM rather than 0.10 mM. For \( \text{KIVDH} \), the optimum was at 0.10 mM. This phenomenon is also being further investigated.

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