Encephalopathy of Thiamine Deficiency: Studies of Intracerebral Mechanisms

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ABSTRACT Thiamine-deficient encephalopathy is characterized by morphologic lesions in the brainstem and less extensively in the cerebellum, but the early neurologic signs reverse rapidly and fully with thiamine, indicating a metabolic disorder. The suggested causal mechanisms of the encephalopathy involve two thiamine-dependent enzymes: (*a*) impairment of pyruvate decarboxylase activity with decreased cerebral energy (ATP) synthesis, and (*b*) reduction of transketolase activity with possible impairment of the hexose monophosphate shunt and subsequent decrease in NADPH formation. The latter may be important in maintaining glutathione in a reduced form (GSH), which apparently functions by keeping enzymes in a reduced (active) conformation.

To examine some of these postulated mechanisms, in this study we measured pyruvate decarboxylase and transketolase activity, lactate, ATP and GSH levels in the cerebral cortex, cerebellum, and brainstem, and thiamine concentration in whole brain of rats with diet-induced low thiamine encephalopathy. Pair-fed and normally fed asymptomatic control animals were similarly investigated. To assess the functional importance of some of our results, we repeated the studies in rats, immediately (16–36 hr) after reversal of the neurological signs with thiamine administration.

The data obtained led to the following conclusions: (*a*) Brain contains a substantial reserve of thiamine in that thiamine level has to fall to below 20% of normal before the onset of overt encephalopathy and an increase in brain thiamine to only 26% of normal results in rapid reversal of neurologic signs. (*b*) Both cerebral transketolase and pyruvate decarboxylase activities are impaired in low thiamine encephalopathy and the abnormality in the pyruvate decarboxylase is reflected in a rise in brain lactate. These biochemical abnormalities occur primarily in the brainstem and cerebellum, the sites of the morphologic changes. (*c*) Although the fall in cerebral transketolase is about twofold greater than that of pyruvate decarboxylase activity during encephalopathy, both enzymes rise on reversal of neurologic signs and the degree of the transketolase rise is slight. Accordingly, this study cannot ascertain the relative functional importance of these two pathways in the induction of the encephalopathy. The data suggest, however, that the depression of transketolase is not functionally important per se, but may only be an index of some other critical aspect of the hexose monophosphate shunt. (*d*) The normal cerebral ATP concentration and small GSH fall during encephalopathy, with little GSH rise on reversal of neurologic signs, suggest that a depletion of neither substance is instrumental in inducing thiamine-deficient encephalopathy.

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INTRODUCTION

Thiamine deficiency in man may induce Wernicke's disease (1, 2) and in experimental animals causes

a similar type of encephalopathy consisting of ataxia, loss of righting, opisthotonos, and drowsiness (3, 4). Both conditions are characterized by morphologic lesions primarily located in the brainstem, and to a lesser extent in the cerebellum, but the early neurologic signs reverse rapidly and fully with thiamine administration suggesting that the encephalopathy is a metabolic disorder (1, 3–5).

Two areas of cerebral metabolism, the tricarboxylic acid cycle (TCA cycle) and the hexose monophosphate shunt (HMP shunt), utilize thiamine-dependent enzymes, and an impairment of these metabolic pathways has been suggested as the mechanism of thiamine-deficient encephalopathy (6). As regards biochemical reactions vital for the TCA cycle, thiamine pyrophosphate is an essential coenzyme of pyruvate decarboxylase, an enzyme that catalyzes the oxidative conversion of pyruvate to acetyl coenzyme A.[1] This high energy compound has a number of biologic functions, of which condensation with oxalacetate to form citric acid, a key initial substrate of the TCA cycle, is perhaps paramount. Another thiamine-dependent enzymatic reaction of the TCA cycle is the decarboxylation of alpha ketoglutarate to succinate, although this step is apparently less affected by thiamine deficiency (7). Thiamine pyrophosphate also serves as a coenzyme for transketolase which catalyzes reactions necessary for the operation of the HMP shunt. The relative importance and functional significance of impaired pyruvate decarboxylation vs. transketolation as a cause of the encephalopathy in thiamine-deficient animals are still unsettled, although most authors currently favor the latter mechanism (9, 10). The subject is discussed extensively in recent publications (9–12).

The manner in which an impairment of the TCA cycle or the HMP shunt may induce the signs of encephalopathy is conjectural. Since, however, the TCA cycle is the principal means of cerebral oxidative synthesis of energy, ultimately in the form of adenosine triphosphate (ATP), it has been postulated that impairment of this pathway by decreased pyruvate oxidation may result in a decrease of available ATP (6, 13, 14).[2] According to current concepts of nerve impulse propagation, such an event may in turn disrupt the orderly integration of cerebral energy-dependent cation flux which is believed essential for normal cerebral function (15, 16). One of the major roles of the HMP shunt is believed to be the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (17). This substance apparently is required to maintain cerebral glutathione in a reduced state (GSH) (18, 42). Since GSH is believed to function by maintaining metabolically important thiol compounds in the reduced form to assure their function as coenzymes and enzymes (18), a decrease in NADPH synthesis may cause a fall in GSH and thus impair cerebral function. This hypothesis is strengthened by the observations that erythtrocyte and heart GSH concentrations were reduced in animals with thiamine deficiency (19). Regional cerebral analyses of ATP, NADPH, and GSH in thiamine-deficient animals have not yet been carried out.

In order to elucidate some of the hypotheses advanced above, this study of cerebral metabolism in rats with thiamine-deficient encephalopathy had the following objectives: (*a*) to determine the relative magnitude of impairment of pyruvate decarboxylase and transketolase activity in various regions of the brain; (*b*) to assess the functional significance of any enzyme impairment noted by reassaying for these immediately after thiamine-induced neurologic recovery; (*c*) to determine if cerebral regional ATP and GSH concentrations are decreased in the encephalopathy of thiamine deficiency; and (*d*) to determine if, as would be anticipated from the morphologic data (4, 5), rat brainstem and cerebellum, as compared to cortex, show a selective biochemical sensitivity (rise in lactate, fall in transketolase and pyruvate decarboxylase activity) to thiamine depletion.

METHODS

Experimental procedure. Sprague-Dawley female littermate rats weighing 50–60 g were individually placed in metabolic cages. One member of each pair was given daily 20 g of a special synthetic thiamine-deficient diet,[3a]

[1] This enzymatic process requires the cooperation of several enzymes and cofactors, and the sum of these has also been designated as pyruvic acid dehydrogenase (8).

[2] This hypothesis implies that cerebral CO_2-fixing reactions in thiamine deficiency are unable to sustain the normal function of the TCA cycle.

[3a] Thiamine deficient diet: sucrose 68%, vitamin-free casein 18%, vegetable oil 10%, salt mixture USP XIV

while the second animal (control) was pair fed with the same diet supplemented with thiamine (control diet).[3b] The daily food intake of the pair-fed control rat depended on the previous day's measured food consumption of the first animal. It has been calculated that 1 g of the control diet provides more than twice the minimum daily requirement of thiamine for rats (9), and the pair-fed controls, although partly starved toward the end of the experimental period, ate at least 1.5 g of the diet per day. A third group of female rats of equal weight was placed on ad lib. regular laboratory diet and served as the "normally fed" control group. All animals were given free access to water. The pair-fed animals were weighed every day and the normally fed rats at least every other day.

The thiamine-deficient rats were sacrificed upon onset of definite neurological signs (described under Results), usually after 4.5–5 wk of thiamine deprivation. Some animals, assayed for brain thiamine, were sacrificed at weekly intervals. Appropriate pair-fed and normally fed control rats were killed at the same time as the corresponding experimental animals. To assess the functional importance of thiamine deficiency on the cerebral processes investigated, we gave other thiamine-deficient rats with overt but brief (duration 24–96 hr) neurologic signs 10 μg of thiamine hydrochloride i.p. Occasionally, a second thiamine injection after 24 hr was required. This resulted in a virtually complete abolition of neurologic signs in all animals within 16–36 hr. Identical thiamine injections were given to the pair-fed controls which were asymptomatic throughout.

The manner in which the animals were sacrificed varied with the purpose of the experiment. For measurement of cerebral regional thiamine, lactate, pyruvate, ATP, GSH, and protein concentrations, and in some instances transketolase activity, the unanesthetized rats were frozen rapidly in a mixture of dry ice and acetone. The frozen brain was then chiseled out and separated into the appropriate regions, while frozen on dry ice, before assay. For measurement of cerebral pyruvate decarboxylase activity, which decreases by 80% on freezing (9), the animals were decapitated, and the unfrozen brain was subdivided into the required areas for assay, while blood was collected from the torso into heparinized tubes for blood transketolase estimation. For greater comparability of results, some cerebral transketolase determinations were also carried out on the same tissues utilized for the pyruvate decarboxylase activity. In some instances pyruvate decarboxylation and transketolase activity were measured in unfrozen liver, kidney, and heart.

The areas of brain assayed consisted of parietal cortical gray matter, the cerebellum, and the brainstem, which comprised mainly the pons but also sometimes included both rostral medulla and caudal midbrain. The approximate landmarks for the brainstem were anteriorly the level of posterior colliculi and caudally the level of the vermis. In five normal animals transketolase and pyruvate decarboxylase activity were also assayed in other regions of the brain (see Results). For heart assay a section of either ventricle, free of blood, was used. Liver tissue was chosen without regard to site. Both cortex and medulla of kidney were assayed.

Methods of analysis. Whole blood transketolase activity and the thiamine pyrophosphate (TPP) effect were measured by the technique of Dreyfus (20). Brain thiamine (combined free and phosphorylated moieties) was assayed by the method of Lewin and Wei (21) in which the thiochrome produced is read fluorometrically. Nonspecific tissue fluorescence was excluded utilizing a tissue blank treated with benzenesulfonyl chloride as described by Haugen (22). Each brain sample was assayed in quadruplicate and the results averaged. Recovery of weighed quantities of thiamine and thiamine pyrophosphate added to whole brain were between 90 and 100% in 10 instances. Lactate was assayed in frozen brain enzymatically and in duplicate by the procedure of Hohorst (23). In a few instances, brainstem pyruvate was estimated enzymatically according to Butcher, Czok, Lamprecht, and Latzko (24). The details and validation of these assays have been presented in a prior publication (25). Cerebral ATP concentration was measured in duplicate in the rapidly frozen brain by the luciferin-luciferase luminescence reaction, as described previously from this laboratory (26). Reduced glutathione (GSH) in brain was estimated in triplicate by the procedure of Beutler, Duron, and Kelly (27). Protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (28).

Cerebral regional, heart, and liver pyruvate decarboxylase activity was measured by the procedure of Dreyfus and Hauser (9). In this assay the decarboxylation of pyruvate-1-^{14}C is estimated from the evolution of $^{14}CO_2$, with the tissue as the source of the decarboxylase. The decarboxylation of pyruvate increased linearly with increase in tissue weight, over the range of tissue weight assayed. The reaction also proceeded at a linear rate during the first 60 min of incubation, with a given brain sample; hence incubation periods of 45 min were used. Each sample was assayed in triplicate.

Cerebral regional, heart, and liver transketolase activity was measured by the method of Dreyfus and Moniz (29). In this procedure excess ribose-5-phosphate is incubated with tissue as the source of transketolase and the rate of sedoheptulose-7-phosphate formation, estimated by a modified Dische technique (29, 30), is determined spectrophotometrically. This method is believed to assess primarily transketolase activity in that the rate of sedo-

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4%; vitamins in g/100 lb. diet—vitamin A 4.5 (200,000 U/g), vitamin D concentrate 9.25 (400,000 U), alpha tocopherol 5.0, ascorbic acid 45.0, inositol 5.0, choline chloride 75.0, menadione 2.25, *p*-aminobenzoic A 5.0, niacin 4.5, riboflavin 1.0, pyridoxine hydrochloride 1.0, calcium pantothenate 3.0, and in mg/100 lb. diet—biotin 20, folic acid 90, vitamin B_{12} 1.35.

[3b] Control diet in addition to the above contained thiamine hydrochloride 1 g/100 lb. diet. Both diets were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

heptulose formation closely parallels ribose utilization, the reaction proceeds linearly over the first 30 min, and sedoheptulose formation both proceeds linearly with increasing concentrations of enzyme (quantity of tissue) and is depressed substantially when tissues from thiamine-deficient animals are employed (29). Since this procedure measures part of a coupled reaction sequence, the possibility that the assay may also reflect other subsequent enzymatic steps has not been wholly eliminated (31). Recent studies (32), however, strongly suggest that the assay accurately measures transketolase activity. Practical assays for tissue transketolase activity by noncoupled micromethods have not been described as yet. In our studies, sedoheptulose elaboration proceeded linearly for 60 min of incubation; hence a 45 min incubation period was chosen. All samples were assayed in triplicate. Recovery of known amounts of sedoheptulose in five instances was between 95–104%. Reproducibility of the assay, when measured in nine contiguous sections of the same cerebral cortex, was within ± 2.5% of the mean. Freezing the brain for at least 1 month did not affect the assay.

Statistical analysis. Most of the data for thiamine-deficient and appropriate pair-fed controls were analyzed by the sign test (33) (for five pairs) or the Wilcoxon matched-pairs signed-ranks test (33) (for more than five pairs of animals). Regional cerebral distribution of pyruvate decarboxylase and transketolase activity was also compared in each animal by these techniques. These statistical tests are ideally suited to assess differences between tissues in matched pairs of animals or between brain areas, when the small number of experiments does not permit one to assume a normal distribution of values. Making such an assumption and utilizing tests such as the *t* test or disregarding the matched pairing and using the nonparametric Mann Whitney U test (33) increased the level of significance in every instance where already observed by the sign test.

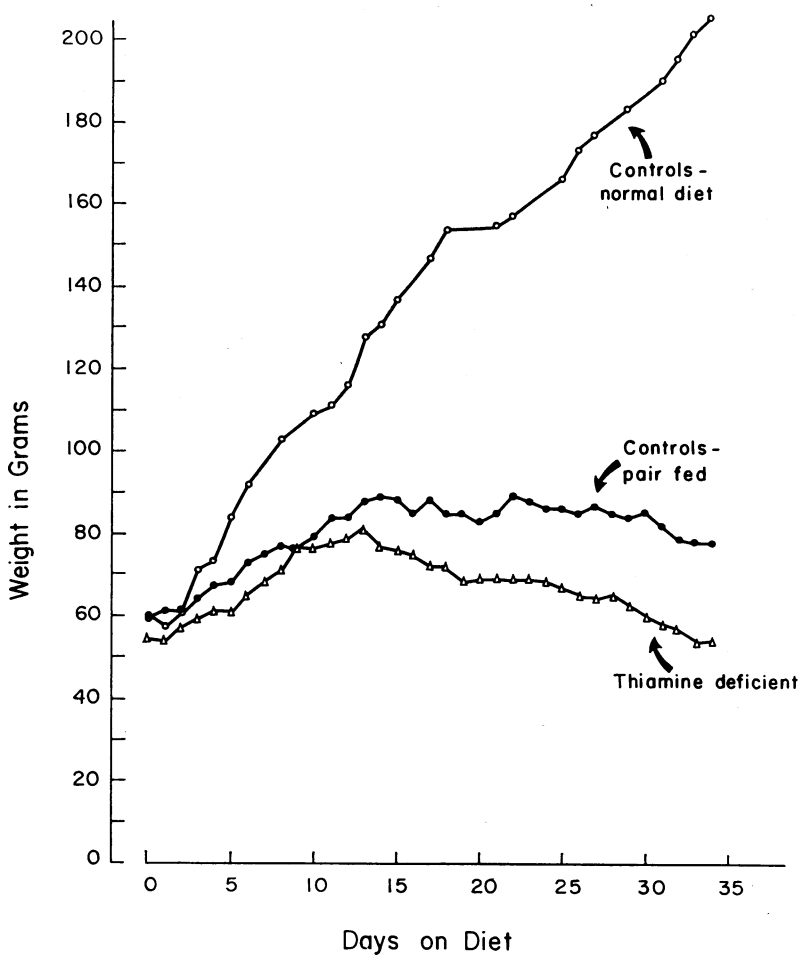

FIGURE 1 The effect of diet-induced thiamine deficiency on the growth of rats. Each point for each group of rats is the mean of three rats. This growth pattern is typical of other groups studied.

RESULTS

Neurologic observations. The weight curve of thiamine-depleted, pair-fed control, and normally fed control rats is shown in Fig. 1. Both control groups remained asymptomatic throughout the course of the experiments. The thiamine-depleted experimental animals reached a peak weight at about 2 wk and thereafter consumed progressively less food and lost weight. At about 2–2.5 wk some hair loss became evident, but the animals appeared neurologically intact until 4.5 wk of low-thiamine diet. Thereafter they developed in rapid progression (1–4 days) slight incoordination on walking, slight impairment of righting reflex, reluctance to walk, walking backward or in circles, severe imbalance on any locomotion, opisthotonic posturing, virtually total loss of righting activity, rolling movements on being picked up by the tail, and drowsiness. Convulsions were not seen. These findings are very similar to those described by Dreyfus for rats placed on the same diet (4). Previous studies have shown that these neurologic signs are the result of central nervous system dysfunction and not of peripheral neuropathy (3). An i.p. injection of 10 μg of thiamine hydrochloride, in a few instances repeated at 24 hr, resulted in reversal of the symptomatic animals to an apparently normal or near-normal neurologic state within 16–36 hr.

Brain thiamine. As shown in Table I, the mean brain thiamine concentration in the normally fed control rats was 3.72 μg/g of brain and this was not significantly diminished in the pair-fed controls over 4.5 wk. This finding then attests to the adequacy of the control diet, in the amounts consumed, in maintaining a normal brain thiamine concentration. By contrast, the rats placed on the thiamine-deficient diet lost 15.1, 43.8, 59.1, 73.1, and 84.3% of control brain thiamine concentration after 1, 2, 3, 3.5, and 4.5 wk, respectively. Significantly, however, the brain thiamine concentrations had to be reduced to less than 20% of normal before onset of neurologic signs and a reversal of the animals to a normal neurologic state occurred with the brain thiamine rising to only 26% of normal.

Pyruvate decarboxylase and lactate. Table II A shows the regional cerebral distribution of pyruvate decarboxylase activity in normally fed control rats. The lowest activity was noted in the brainstem and the highest in the cerebellum. Similar findings were seen in the pair-fed controls (Table III). In five normal rats the mean pyruvate decarboxylase activity in subcortical white matter

TABLE I

Whole Brain Thiamine Concentration in Thiamine-Deficient Rats

					4½–5 wk	
Duration of experiment....	1 wk	2 wk	3 wk	3½ wk	Symptomatic* stage	Reversed* stage
Pair-fed controls‡	3.81 ± 0.09§ (3)	4.02 ± 0.10 (3)	3.80 ± 0.17 (3)	3.53 ± 0.07 (3)	3.56 ± 0.15 (5)	3.54 ± 0.11 (10)
Thiamine deficient	3.21 ± 0.11 (3)	2.26 ± 0.07 (3)	1.55 ± 0.06 (3)	0.95 ± 0.05 (3)	0.56 ± 0.07 (5)	0.91 ± 0.06 (10)
% Decrease from control	15.7‖	43.8‖	59.1‖	73.1‖	84.3¶	74.3**

* Symptomatic and "reversed" stages are described in the text under Results.

‡ Brain thiamine concentration in 7 normal controls, not pair fed, was 3.72 ± 0.08 μg/g. This value is not significantly different from results obtained in the pair-fed controls ($p > 0.05$) (33).

§ Mean ± SE of number of animals indicated in parentheses. The results are given in μg/g whole brain, wet wt. Each determination represents the mean of quadruplicate assays.

‖ Because of the small sample, statistical analysis was carried out by paired t test which assumes a normal distribution. The values in thiamine-deficient animals at 1 wk were not significantly decreased whereas at 2, 3 and 3.5 wks the brain thiamine was significantly ($P < 0.01$) lower.

¶ Significant at $p < 0.04$. Data analyzed by nonparametric sign test (33) (see Methods.)

** Significant at $p < 0.001$ (33).

TABLE II

Regional Cerebral Pyruvate Decarboxylase and Transketolase Activity in Normal Rats

	Cortex	Cerebellum	Brainstem
A. Pyruvate decarboxylase activity			
μmoles pyruvate decarboxylated /45 min per g brain wet wt	132.9 ± 10.3*	163.3 ± 14.3	106.4 ± 9.1
μmoles pyruvate decarboxylated /45 min per mg brain protein	1.18 ± 0.15	1.41 ± 0.15	9.92 ± 0.13
B. Transketolase activity			
μmoles sedoheptulose /45 min per g brain wet wt	11.63 + 1.01*	21.52 ± 1.18	18.97 ± 1.53
μmoles sedoheptulose /45 min per g brain protein	65.5 ± 6.1	123.1 ± 8.0	116.3 ± 9.9

* Mean ± SE of five rats. Each brain sample was assayed in triplicate for the decarboxylase and in quadruplicate for the transketolase measurements and the results were averaged.

For the decarboxylase study the differences among the three brain areas are all statistically significant ($p < 0.04$), both when expressed per g brain wet wt and per mg brain protein.

For the transketolase study, on the basis of tissue wet wt and protein the cortical value is significantly ($P < 0.04$) below both the cerebellar and brainstem values which are comparable ($P > 0.05$).

Statistics were carried out by the nonparametric sign test wherein the brain areas from each animal are compared to each other (33).

was 19.4% below that of the cortex ($P < 0.04$). In the thiamine-deficient symptomatic rats cortical pyruvate decarboxylase activity was not significantly depressed while that in cerebellum decreased by 35.8% and in the brainstem by 28.6% ($P < 0.04$) (Table III A). On reversal of the encephalopathy, the depression of the pyruvate decarboxylase activity in the thiamine-deficient animals in both cerebellum and brainstem was substantially reduced (Table III B). The measure-

TABLE III

Effect of Thiamine Deficiency on Regional Cerebral Pyruvate Decarboxylase Activity

	A. Thiamine deficient symptomatic group			
	Pair-fed controls for symptomatic thiamine deficient group	Thiamine-deficient symptomatic group	% Decrease	*P* value
Cortex	119.9 ± 5.3*	105.1 ± 7.1	12.0	>0.05
Cerebellum	158.9 ± 20.3	96.5 ± 7.9	35.8	<0.04
Brainstem	84.3 ± 7.9	58.6 ± 2.6	28.6	<0.04
	B. Thiamine deficient "reversed" asymptomatic group			
	Pair-fed controls for "reversed" thiamine-deficient group	"Reversed" thiamine deficient group, asymptomatic	% Decrease	*P* value
Cortex	130.9 ± 20.4*	130.0 ± 10.9	0.8	>0.05
Cerebellum	184.1 ± 23.3	148.6 ± 21.4	19.2	<0.04
Brainstem	93.7 ± 5.1	79.8 ± 5.3	15.0	<0.04

* Mean ± SE of five animals in each group. All values are the means of triplicate assays. The results are expressed in μmoles of pyruvate decarboxylated/45 min per g brain wet wt. The symptomatic and "reversed" groups are defined in Results.

TABLE IV

Effect of Thiamine Deficiency on Regional Cerebral Lactate Concentration

	Cortex	Cerebellum	Brainstem
Normal control	16.53±1.76* (6)	27.79±2.18 (12)	27.49±3.42 (12)
Pair-fed control for symptomatic thiamine-deficient group	23.70±1.80 (9)	28.03±2.54 (9)	25.78±1.81 (9)
Thiamine-deficient symptomatic‡	25.43±1.78 (9)	37.01±4.08§ (9)	43.53±5.02‖ (9)
Pair-fed control for "reversed" thiamine-deficient group	20.62±3.90 (5)	—	22.06±1.49 (5)
Thiamine-deficient "reversed"‡	21.87±2.56 (5)	—	31.75±3.27¶‡ (5)

* Mean ± SE of number of animals given in parentheses. Values expressed in mg/100 g brain, wet wt. From assay of blood lactates in a smaller group of control and thiamine-deficient animals and from data on brain blood content (25), it was calculated that correction of brain lactate for the trapped blood lactate would not significantly alter the values given above, hence such a correction was not carried out.

‡ Symptomatic and "reversed" groups defined in Results.

§ Significantly higher than appropriate pair-fed control group ($P < 0.03$).

‖ Significantly higher than appropriate pair-fed control group ($P < 0.01$).

¶ Significantly higher than appropriate pair-fed control group ($P < 0.04$).

ments of cerebral regional lactate concentrations (Table IV) were generally consistent with the above considerations. Thus, in the thiamine-deficient symptomatic animals cortical lactate was normal while lactate in both cerebellum and brainstem rose significantly by 32 and 68.9%, respectively ($P < 0.03$). The lack of quantitative correlation between the degree of pyruvate decarboxylase depression and lactate rise in these two regions may not be surprising since different sets of animals were used for each assay. On reversal of neurologic signs brainstem lactate in the thiamine-deficient animals was still elevated, though only by 43.9% ($P < 0.04$), while the cortical value remained unaltered (Table IV). In a few instances brainstem pyruvate concentration was measured in pair-fed and thiamine-deficient symptomatic rats. The pyruvate concentration in the symptomatic animals was invariably elevated and the degree of rise paralleled that observed with lactate measurements. Pyruvate decarboxylase activity in liver, heart, and kidney cortex of the thiamine-deficient symptomatic animals was depressed 68, 80, and 52%, respectively, below that noted in pair-fed controls ($P < 0.04$).

ATP. Table V depicts the regional cerebral ATP concentration in normally fed and pair-fed controls as well as thiamine-deficient symptomatic rats. In all areas of the brain studied the ATP values in the animals with various stages of low thiamine encephalopathy were comparable to those of the asymptomatic controls ($P > 0.05$).

Transketolase. The regional cerebral distributions of transketolase activity in normally and pair-fed control rats are shown in Tables II and VI, respectively. The data for both groups of animals are quite similar and confirm the studies of Dreyfus (39) in showing a significantly lower transketolase activity in the cerebral cortex than in the cerebellum and brainstem. Cortical samples obtained from the frontal, parietal, and occipital cortex in five normal rats gave almost identical results. Transketolase activity in the cerebellar hemispheres and vermis was also similar ($P > 0.05$) and comparison of medulla, lateral pons, medial pons, and midbrain in five rats likewise demonstrated no differences ($P > 0.05$) among these areas. Furthermore, transketolase activity in the cortex and subcortical white matter of five other normal rats was virtually identical for each animal ($P > 0.05$) both on the basis of wet weight, dry weight, and tissue protein concentration. In the thiamine-deficient rats a significant depression of transketolase activity was seen in the cortex, cerebellum, and brainstem, in that order of increasing magnitude (Table VI, group 1). Whole blood, heart, and liver transketolase in the same symptomatic animals was decreased by 88.2, 65.8,

TABLE V

Effect of Thiamine Deficiency on Regional Cerebral ATP Concentration

	Cortex	Cerebellum	Brainstem
Normal controls (6)	2.69±0.04*	2.40±0.03	1.92±0.10
Pair-fed controls for thiamine-deficient group (10)	2.49±0.07	2.51±0.05	2.02±0.06
Thiamine-deficient moderate‡ (6)	2.52±0.06	2.50±0.11	2.02±0.06
Thiamine-deficient severe‡ (4)	2.46±0.02	2.59±0.12	2.00±0.11

* Mean ± SE. Values expressed in μmoles/g brain, wet wt. Number of animals used is shown in parentheses. None of the values in any one area of the brain are significantly different.

‡ Animals showing moderate loss of righting reflex and incoordination on walking were termed moderate. Those exhibiting opisthotonos, virtually total loss of balance and drowsiness, were termed severe (see Results).

TABLE VI

Effect of Thiamine Deficiency on Regional Cerebral Transketolase Activity

		Pair-fed controls for symptomatic thiamine-deficient group	Thiamine-deficient symptomatic group	% Decrease	*P* value
Group 1‡ (5)	Cortex	11.78 ± 1.49*	6.56 ± 0.66	44.3	<0.04
	Cerebellum	22.09 ± 2.15	9.68 ± 1.25	56.2	<0.04
	Brainstem	19.66 ± 3.40	6.94 ± 0.73	64.7	<0.04
Group 2‡ (5)	Brainstem	11.94 ± 2.50	4.58 ± 0.31	61.6	<0.04
		Pair-fed controls for "reversed" thiamine-deficient group	"Reversed" thiamine-deficient asymptomatic group	% Decrease	*P* value
Group 3‡ (7)	Brainstem	12.56 ± 1.01	5.81 ± 0.45	53.7	<0.01

* Mean + SE of the number of animals shown in parentheses for each group. Each section of brain is the mean of triplicate determinations. The values are expressed as μmoles sedoheptulose/45 min per g brain wet wt.

‡ Group 1 refer to animals sacrificed by freezing in dry ice and acetone. Groups 2 and 3 refer to other litters of rats sacrificed by decapitation. The pyruvate decarboxylase activity (Table III) was measured in the same brains utilized for the transketolase assay in groups 2 and 3. Pyurvate decarboxylase is decreased by freezing of the tissues, (9) hence the need for a variation in experimental technique.

and 55%, respectively ($P < 0.04$). In the kidney cortex and medulla of the symptomatic animals transketolase activity decreased by 52 and 80%, respectively ($P < 0.04$). The TPP effect on blood transketolase in the thiamine-deficient rats was 77.5%, as compared to 13% in the pair-fed controls. In the second group of animals (Table VI), killed by decapitation and employed also for assay of pyruvate decarboxylase activity (Table III), a very similar degree of fall in brainstem transketolase activity occurred in the symptomatic rats. The differences in the absolute values for brainstem transketolase between groups 1 and 2, sacrificed by freezing or decapitation, respectively (Table VI), may represent variation between animal groups, method of sacrifice, or both. It is evident, however, from comparison of the data in Tables III and VI, that the transketolase fall was about 2.5 times greater than that for pyruvate decarboxylase activity. Surprisingly, on reversal of neurologic signs, mean brainstem transketolase in seven animals was still depressed by 53.8% (Table VI, group 3). This then represents a smaller improvement, as compared to controls, than was noted in the same animals for the brainstem pyruvate decarboxylase activity (Table III).

GSH. As shown in Table VII, the concentration of GSH in the brain of normally fed rats was slightly higher than in that of the pair-fed controls. This presumably reflects the effect of semi-starvation in the latter group. In the symptomatic thiamine-depleted rats cortical and cerebellar GSH concentration was comparable to that noted in the

TABLE VII

Effect of Thiamine Deficiency on Regional Cerebral Glutathione Concentration*

	Cortex	Cerebellum	Brainstem
Normal control (6)	636.1±12.3‡	625.0±29.0	512.4±15.0
Pair-fed controls for symptomatic thiamine-deficient group (6)	579.1±12.6	529.5±25.8	465.3±24.4
Thiamine-deficient symptomatic group (6)	553.1±22.5	519.1±21.4	382.3±10.2§
Pair-fed controls for "reversed" thiamine-deficient group (8)	—	—	447.5±22.1
Thiamine-deficient "reversed" group (8)	—	—	387.2±11.9§

* Glutathione refers to the reduced moiety, GSH.

‡ Mean ± SE. Values expressed in mg/100 g brain, wet wt. Numbers of animals used are shown in parentheses.

§ Significantly lower than appropriate pair-fed control value ($P < 0.03$) (33).

asymptomatic pair-fed controls while brainstem GSH was lower by only 18% ($P < 0.03$). On reversal of neurologic signs brainstem GSH in the thiamine-deficient animals was still lower by 13% ($P < 0.03$).

Protein. Protein concentrations in cortex, cerebellum, and brainstem of five sets of thiamine-deficient symptomatic rats and in the same areas in five pair-fed controls were comparable ($P < 0.05$), justifying the expression of the results in terms of tissue wet weight.

DISCUSSION

The present study defines the critical range of brain thiamine concentrations necessary for the induction and the subsequent resolution of neurologic signs in rats with diet-induced thiamine deficiency. Contrary to some published data (34, 35), whole brain thiamine in the experimental animals began to fall at 1 wk and decreased by 44% after 2 wk on a thiamine-deficient diet (Table I), despite lack of neurologic signs and a growth comparable to that of the pair-fed control animals (Fig. 1) which maintained a normal brain thiamine concentration. Furthermore, the whole brain thiamine concentration had to be reduced to less than 20% of normal before the onset of overt encephalopathy and a reversal of the animals to an essentially normal neurologic state occurred with the brain thiamine rising to only 26% of normal (Table I). Subtle neurologic changes may occur with a somewhat lesser depletion of thiamine (3). These results are quite comparable to the data of Dreyfus (4) and of Balaghi and Pearson (36), and indicate both that brain thiamine does decrease in the early stages of a thiamine-deficient state, and that brain contains a substantial reserve of thiamine, in that only a severe depletion of the vitamin to less than four-fifths of normal results in a neurologic dysfunction. The validity of these observations is reinforced by available data that during the induction of a thiamine-deficient state the whole brain thiamine level accurately reflects its regional concentration (8) and its subcellular distribution (36). Furthermore, the rapid reversal of the encephalopathy with thiamine administration, of course, confirms the metabolic origin of this disorder.

There has been considerable discussion whether a significant depression of cerebral pyruvate decarboxylase activity occurs in the encephalopathy of thiamine deficiency. In the classical studies of this "biochemical lesion," Peters demonstrated impaired oxidation of pyruvate and lactate by brain of thiamine-deficient pigeons and rats in vitro and an accumulation of pyruvate and lactate in the brain of these animals (37). In the thiamine-deficient pigeons the morphologic and biochemical alteration were most striking in the brainstem whereas in the rats a definite localization of the biochemical defect was not obtained (37, 38). In these earlier investigations, the brain pyruvate and lactate estimations were necessarily carried out by nonspecific techniques. Subsequently, Gubler using whole brain (7) and Dreyfus with brainstem, but not cortex or cerebellum (8), found a statistically significant decrease in pyruvate decarboxylase activity in rats showing neurologic signs of thiamine deficiency. Both authors, however, felt that the decrease in this pathway was too small, especially as compared to the fall in brainstem transketolase activity (39), to qualify as an important cause of the encephalopathy. These latter arguments were reiterated by some investigators who found a normal oxidation of infused labeled pyruvate to $^{14}CO_2$ in thiamine-deficient rats (10) and others who postulated a normal decarboxylation of pyruvate to acetyl-CoA in these animals on the basis of the isotopic pattern of cerebral glutamate after administration of labeled glucose (11).

The present study confirms the observations of Dreyfus and Hauser (9) that pyruvate decarboxylase activity is significantly decreased in the brainstem of rats with low thiamine encephalopathy, while the enzyme in the cortex is unaltered. In addition, these studies (Table III) show that cerebellar pyruvate decarboxylase activity in the symptomatic animals is also depressed by about 35% ($P < 0.04$). This demonstration of impaired pyruvate entry into the TCA cycle in these two brain areas is supported by the observations that lactate, and in a few instances pyruvate, measured by specific enzymatic techniques, also accumulated in these two sites, but not in the cortex of the thiamine-deficient rats with neurologic signs (Table IV).[4] This localization of these biochemical

[4] This interpretation assumes that the abnormal brain lactate and pyruvate concentration in thiamine deficiency

abnormalities is consistent with the observations that morphologic lesions in these animals also are present in the brainstem and cerebellum, with sparing of the cerebral cortex (4, 5).

It is not possible from currently available data to evaluate conclusively the *functional* importance of impaired cerebral pyruvate decarboxylation, as compared to decreased transketolase, for the induction of the neurologic dysfunction seen in the thiamine-deficient animals. First, a normal elaboration of $^{14}CO_2$ from labeled pyruvate injected into thiamine-deficient animals (9) depends on the over-all pyruvate decarboxylation in various tissues, and may not necessarily reflect regional cerebral metabolism of pyruvate. Second, the previously cited study (10), based on differential labeling of cerebral glutamate after infusion of glucose-^{14}C, was carried out assaying only whole brain. Finally, although Dreyfus observed an earlier and greater fall in brainstem transketolase than pyruvate decarboxylase activity in thiamine-deficient rats (9) and logically related the transketolase fall to the observed morphologic abnormalities in oligodendrocytes and myelin in the brainstem of these animals (12, 14), these observations do not rule out the possibility that a small but perhaps critical depletion of the pyruvate decarboxylase pathway may be of considerable significance in the induction of the low thiamine encephalopathy.

In an attempt to delineate more precisely the relative importance of these two enzymatic pathways in inducing low thiamine encephalopathy, we compared regional cerebral transketolase and pyruvate decarboxylase activity first in rats with overt encephalopathy, and then in other animals immediately after rapid reversal of their neurologic signs with thiamine. Inasmuch as the recovery from encephalopathy ensued within only 16–36 hr after administration of the thiamine, it was hoped that the activity of one of the enzymes, more likely the pyruvate decarboxylase, might remain fully abnormal allowing one to disregard it as a primary factor in the onset of the encephalopathy. Our studies in rats with overt encephalopathy corroborate those of Dreyfus (9, 39) in that (*a*) transketolase activity is significantly depressed in all areas of the brain studied (Table VI), (*b*) the greatest decrease occurs in brainstem and the least in the cortex, and (*c*) the brainstem transketolase depletion is about 2.5-fold greater than pyruvate decarboxylase. This strikingly greater depression of transketolase than of pyruvate decarboxylase activity in the thiamine-deficient rats is apparently unique for the brain since the decrease in both enzymes was quite similar in liver, heart, and kidney (see Results). Surprisingly, immediately on reversal of the neurologic signs, pyruvate decarboxylase activity increased substantially toward normal in both brainstem and cerebellum (Table III). This improvement in the metabolism of pyruvate was reflected in a similar decrease in brainstem lactate on recovery (Table IV), which, however, still remained significantly elevated as compared to normal. In contrast to the effect of "reversal" on cerebral pyruvate decarboxylase activity, brainstem transketolase in these asymptomatic animals rose very little from the values obtained in rats with overt encephalopathy (Table VI). As a matter of fact, although the mean brainstem transketolase activity in the "reversed" group was decreased by 53.7% from normal vs. a 61.6% fall in the symptomatic animals, in four of the seven "reversed" animals the degree of transketolase decrease overlapped with the values obtained in the rats with encephalopathy. These data differ from the admittedly preliminary results of Dreyfus (39), obtained in two animals, which showed a more substantial improvement in brainstem transketolase on reversal of neurologic signs. Inasmuch as *both* pyruvate decarboxylase and transketolase activity in brain rose with reversal of neurologic signs, it is evident that this approach will not allow a differentiation of the relative importance of these individual pathways in the genesis of the encephalopathy. The very small rise in transketolase on recovery from encephalopathy does not rule out the possible significance of this pathway since a small increase in a critical substrate or enzyme, as shown clearly for brain thiamine (Table I), may reverse neurologic dysfunction and since the depression of transketolase could not be determined in the same animal during encephalopathy and after recovery from it. The data here presented, however, revive the possible importance of the pyruvate decarboxylating pathway as a factor in low thiamine encephalopathy

represents an impaired utilization of these substances and not an alteration in their synthesis, i.e. increased glycolysis, for which there is at present no evidence.

and conversely raise some question concerning the *functional* significance of the depressed brain transketolase per se. One may speculate that the latter enzyme rather serves as an index of other, as yet undefined, HMP shunt-dependent reactions which on specific testing may show much greater change on recovery from encephalopathy, and which may have been responsible for its induction.

Pyruvate oxidation in the TCA cycle is the main source of energy, ultimately in the form of ATP, for cerebral function (6). It is therefore generally postulated that if impaired pyruvate decarboxylation is an important factor in the initiation of low thiamine encephalopathy, this effect is mediated via decreased synthesis, resulting in reduced availability, of cerebral ATP (6, 13, 14). The present study suggests that this concept is incorrect, since in all areas of the brain studied, the ATP concentrations in the symptomatic thiamine-deficient animals were normal (Table V). Current methods do not permit assessment of the rates of synthesis and utilization of regional cerebral ATP in vivo, and these data refer to the net available cerebral ATP. However, since the ATP values were normal at various stages of neurologic dysfunction, this either means that (*a*) the high energy phosphate was synthesized normally, (*b*) that its utilization in brain was impaired exactly equally to a decreased rate of its formation, or (*c*) that both synthesis and utilization were equally increased. There are at present no theoretical or factual data to implicate abnormal cerebral ATP utilization in thiamine deficiency and it seems most reasonable to suggest that the normal cerebral ATP levels are the result of its normal synthesis. This study, therefore, indicates that if impaired pyruvate decarboxylation is a significant cause of the encephalopathy of thiamine deficiency, this effect is not mediated via decreased cerebral ATP and other mechanisms should be investigated.

The importance and role of the HMP shunt in cerebral metabolism and the effect of decreased transketolation on its function are still undefined, although it is believed that this pathway is most active in the oligodendroglia of white matter (12, 40, 41). With regard to the possible mechanism by which an impaired HMP shunt in thiamine deficiency may induce neurological signs, most attention has focused on a decreased synthesis of NADPH (13). Such an abnormality, if present, might impair fatty acid synthesis which may be essential for maintenance of normal brain myelin (17). Alternately, diminished NADPH may impair the conversion of oxidized glutathione to its reduced form (GSH) which is believed to maintain metabolically important compounds in their reduced, i.e. active form (18, 19). In this study, regional cerebral GSH measurements were carried out in normally and pair-fed controls as well as in the thiamine-deficient rats with encephalopathy and after reversal of the neurologic signs (Table VII). The pair-fed asymptomatic controls had lower GSH concentrations than the normally fed animals in each area of the brain assayed. This presumably reflects the state of semi-starvation in the former group. By contrast, the thiamine-deficient symptomatic animals, as compared to the pair-fed controls, manifested a statistically significant depression of GSH only in the brainstem. One may tentatively postulate that this reflects the selectively greatest impairment of the HMP shunt and NADPH production in this brain area, a hypothesis which needs to be confirmed by directly measuring the NADPH/NADP concentrations. Regardless of cause, it is evident that the decrease in brainstem GSH in the symptomatic animals, although statistically significant, was quite small and to a large extent persisted after reversal of neurologic signs. It seems most likely, therefore, that the small brainstem GSH decrease during encephalopathy is a manifestation of the underlying dysfunction in the HMP shunt and is not of itself a cause of the neurologic signs. This interpretation, as well as those concerned with brain ATP and the enzyme assays, presumes that more significant alterations in these substances do not occur in some vital localized cerebral areas or subcellular pools, not assayed in this study,

Our data also provide information on the distribution of transketolase and pyruvate decarboxylase activity in normal rat brain. In agreement with the data of Dreyfus (9, 39), normal brain transketolase activity is higher in brainstem than in the cortex (Table II B) and the opposite is true for pyruvate decarboxylase activity (Table II A) whether expressed in terms of tissue wet weight or protein. Pyruvate decarboxylase activity of cortex likewise exceeds that of the subcortical white matter. These results suggest that transketolase denotes primarily the HMP shunt of white matter

and pyruvate decarboxylase correlates with neuronal metabolism of gray matter. This hypothesis, however, is clouded by the observations that rat, and apparently human (39), subcortical white matter and cortical gray tissue exhibit a similar transketolase activity (see Results) and that the activity of this enzyme in rat cerebellum (a composite of white and gray matter) is equal to that of the brainstem (Table II B). Similarly, pyruvate decarboxylase activity in the cerebellum significantly exceeded that of the cortex (Table II A). These data, therefore, suggest rather that various areas of the brain exhibit their own intrinsic enzymatic activity, at least for these two enzymes and in the sites assayed, and that this activity correlates only in part with the distribution of white and gray matter.

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