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THIAMIN AND THE BRAIN

Richard H. Haas

Departments of Neurosciences and Pediatrics, University of California, San Diego, La Jolla, California 92093

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INTRODUCTION

Thiamin (vitamin B_1) was first isolated in 1926 (1) as the anti-beriberi factor. It was synthesized in 1936 (2). The thiamin deficiency disease beriberi was

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thought of as a disease of the peripheral nervous system, heart, and muscles until the mid-1930s when it was realized that Wernicke's encephalopathy was the acute cerebral manifestation of severe thiamin deficiency. Beriberi remains a major problem worldwide in underdeveloped countries, and the Wernicke-Korsakoff syndrome is a common consequence of alcoholism in the developed world.

Brain thiamin is mostly present as the phosphorylated diphosphate (TDP) and in this form it acts as a cofactor for two key enzyme complexes involved in oxidative metabolism, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. The role of thiamin triphosphate (TTP) remains enigmatic, although evidence is emerging that this form of the vitamin is involved in nerve membrane function.

This review summarizes current information on the role of thiamin in brain function. Although the vitamin was synthesized over 50 years ago and has been intensively studied since, much remains unclear about the cellular and molecular mechanisms of thiamin action in the brain.

HISTORY OF THIAMIN

In his *De Medicina Indorum,* Jacob Bontius in 1645 first drew the attention of Western medicine to the disease beriberi (3). Thiamin deficiency remains a significant problem in much of the developing world. The Interdepartmental Committee on Nutrition for National Defense (ICNND) reports in 1960 on Vietnam (4) and 1962 on Thailand (5) found subclinical and clinical thiamin deficiency persisting in rural areas, with beriberi accounting for 7.7 deaths per 100,000 population in Thailand in 1957. In the Philippines (6) and in India (7) beriberi is also primarily a rural disease in rice-growing areas where polished milled rice provides the major food staple. Early in the 20th century beriberi was a disease particularly of imported Chinese laborers who combined heavy manual labor with a thiamin-deficient diet (8, 9). From the early part of the 20th century infantile beriberi has been a significant cause of mortality among rice-eating populations. In recent years thiamin deficiency has become less of a problem in the western Pacific region (82). However, beriberi continues to be recognized in breast-fed infants of mothers who combine a dietary deficiency with the stress of pregnancy (6, 7). There is evidence that subclinical thiamin deficiency may be much more common than previously thought even in Western developed countries (10–12) and Japan (13). Alcohol-related thiamin deficiency is the third commonest cause of dementia in the United States (14).

The earliest descriptions of beriberi noted the wet and dry forms. Cardiomyopathy and a high output state lead to the edema of wet beriberi. Dry beriberi is the neuropathic form of thiamin deficiency in which peripheral

neuropathy predominates. Beriberi was considered an infectious or toxic disorder for several years after Eijkmen (15) noted its association with a milled rice diet. His fowl model displayed both cardiac and neuropathic pathology. Replacing the milled with raw whole-grain rice rapidly cured the birds, and the rice polishings contained the curative factor.

An excellent account by Williams (16) reviews the early work linking human berberi to deficiency of an essential nutrient. In 1907 Fletcher (44) reported a remarkable prospective controlled human experiment demonstrating that a diet of parboiled rice prevented beriberi whereas a white polished rice diet did not. In a series of human and animal studies published from 1909 to 1915, Frazier & Stanton (cited in 16) showed that human beriberi was a deficiency disease that responded to extract of rice polishings. Chamberlain et al (17) experimented further with the fowl polyneuritis model and the curative effects of extract of rice polishings. The anti-beriberi factor was isolated in 1926 by Jansen & Donath (1). After a quarter of a century of work by Williams (cited in 9) the synthesis of thiamin was accomplished in 1936 (2). The role of thiamin in pyruvate metabolism of brain was identified by the pioneering work of Peters, who coined the term "biochemical lesion" to describe the failure of thiamin-deficient pigeons to metabolize pyruvate (18, 19).

As in peripheral forms of beriberi, the true nature of Wernicke's encephalopathy was unsuspected for many years. The first description in 1881 reported an acute illness with ataxia, mental disturbance, and eye movement paralysis in three patients who later died (20). The Wernicke-Korsakoff syndrome comprising Wernicke encephalopathy and Korsakoff psychosis may be considered the cerebral form of beriberi (21). The brain lesions in thiamin-deficient pigeons were noted to be similar to Wernicke lesions in 1938 (22), pointing the way to thiamin treatment of this devasting disorder.

BIOCHEMISTRY AND METABOLISM

Biosynthesis

Thiamin synthesis occurs in a variety of microorganisms and plants. Synthetic ability in higher animals has not been proven as microorganisms residing in the gastrointestinal tract may be responsible for apparent thiamin synthesis in rats and pigeons. Gut bacterial activity in ruminants can supply the whole thiamin requirement (23).

Thiamin is composed of pyrimidine and thiazole moieties linked by a methylene bridge. Its biosynthesis requires phosphorylation of the precursors before synthesis of the thiamin monophosphate (TMP) molecule (Figure 1). The complex steps involved in synthesis have recently been reviewed (24). Different pathways exist in yeast and bacteria for the synthesis of the pyrimi-

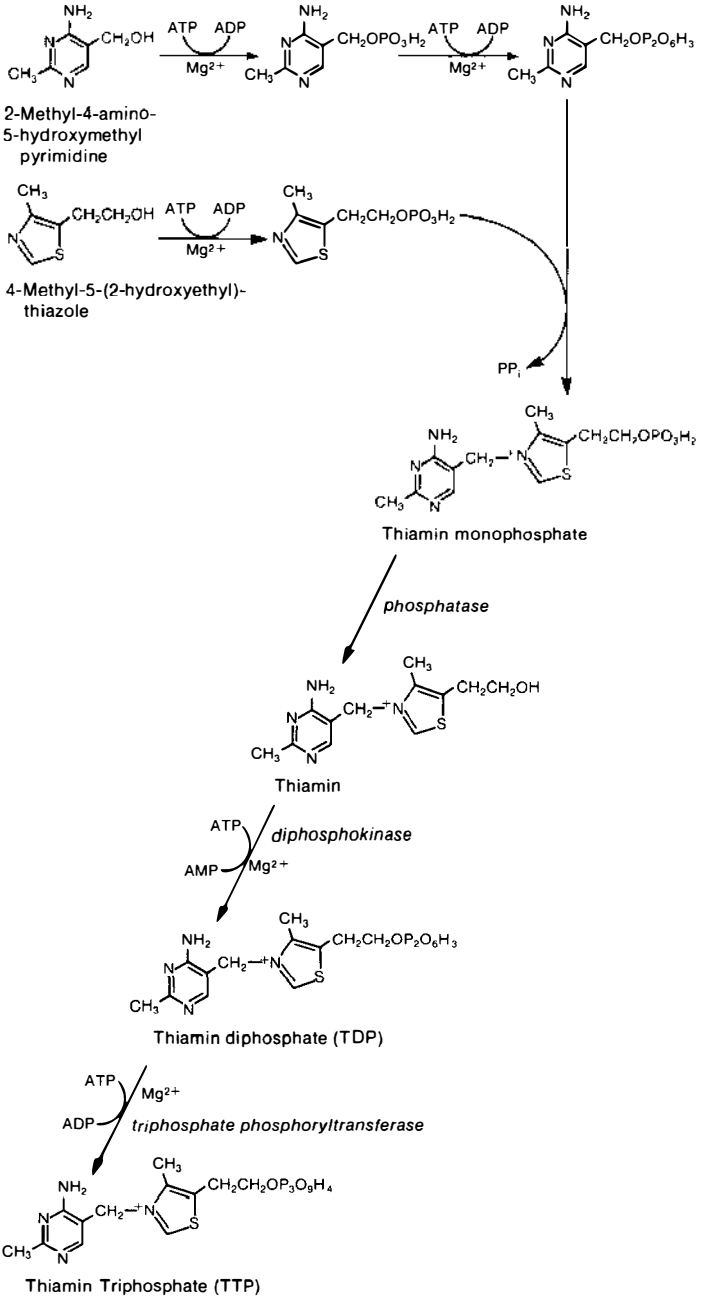

Figure 1 Biosynthesis of thiamin and its phosphorylated derivatives. The initial pathway to thiamin monophosphate has not been shown to occur in higher animals.

dine and thiazole precursors, but the enzyme for the final step synthesizing thiamin monophosphate (thiamin phosphate diphosphorylase) has been purified from yeasts (25) and *E. coli* (26). Thiamin diphosphokinase, responsible for the synthesis of thiamin diphosphate from the free vitamin, has been found in yeast (27, 28) and mammalian tissues (29). *E. coli* can synthesize the diphosphate from the monophosphate (30, 31). The properties of thiamin diphosphokinase have been studied in pig brain (32). The thiamin antimetabolites, oxythiamin and pyrithiamin, inhibit the pig brain enzyme with K_i of 8.9×10^{-5} and 2.2×10^{-7} M, respectively (32).

Thiamin triphosphate is synthesized in brain by the action of a TDP-ATP phosphoryltransferase. However, studies of ^{14}C thiamin turnover show very little incorporation of lable into TTP (33). This enzyme was identified in rat brain mitochondria (34) and subsequently characterized in rat liver (35). TDP must be bound to an endogenous protein in order to act as a substrate, and free TDP does not affect the reaction (35).

Biochemical Role of TDP

TDP serves as the coenzyme for a large number of enzyme systems. These have been classified into three main groups (36): (*a*) oxidative decarboxylations, (*b*) nonoxidative decarboxylations, and (*c*) phosphoketolase reactions in which the acyl group of a ketose is converted to an acyl phosphate. Nonoxidative decarboxylations and the phosphoketolase reaction appear restricted primarily to microbial metabolism and are not discussed further here. TDP is the coenzyme for the two oxidative decarboxylation reactions related to the citric acid cycle. These are the pyruvate dehydrogenase complex and the α-ketoglutarate dehydrogenase complex. In both cases, the ketoacid is converted to an active acyl moiety and ultimately reacts with coenzyme A.

Thiamin can act in nonenzymatic systems to form acetoin from pyruvate (37). Studies in a number of different enzymatic systems have demonstrated that a substitution on position 2 of the thiazole ring forms the active intermediate α-hydroxyethyl thiamin diphosphate. This intermediate has been shown to act as substrate for the formation of acetaldehyde from pyruvate by wheat germ pyruvic carboxylase, the formation of acetoin from pyruvate by the acetoin-forming complex obtained from *Aerobacter aerogenes,* and the formation of acetate by a simulated pyruvate oxidase reaction using ferricyanide as an electron acceptor (38). In the pyruvate dehydrogenase complex, oxidation of the α-hydroxyethyl group of TDP to an active acetyl group is thought to occur by an electron transfer to oxidized lipoic acid followed by transfer of the acetyl group to dihydrolipoic acid. The glycoaldehyde-TDP intermediate in the transketolase reaction forming sedoheptulose-7-phosphate is the α,β-dihydroxyethyl thiamin diphosphate (38).

BALANCE

Best dietary sources of thiamin include whole-grain and enriched cereals, meat (especially pork), poultry, fish, vegetables, and dairy products. Polished rice, sugar, fat, and many refined foods are poor sources of thiamin. The current recommended daily allowance (RDA) is 1.4 mg per day for males and 1.0 mg per day for females in the age range 23 to 50 years. This corresponds to 0.5 mg per 1000 kcal consumed (39). The effects of food preparation are estimated at a 15% loss in the calculation of the RDA; however, modern refined foods and cooking practices such as prolonged heating may reduce available thiamin by as much as 40%. The adequacy of individual intakes can therefore not be accurately estimated from raw food thiamin content. Clinical observations spanning several decades have confirmed the role of a high-calorie and in particular high-carbohydrate diet in increasing thiamin requirement. Physical exertion and high calorie intake are common precipitants of acute beriberi (40).

Brain Content

Total body content of thiamin in the adult human is approximately 30 mg. Numerous early studies of thiamin content in various rat tissues found levels in heart, kidney, and liver to be 2 to 3 times greater than in brain (41). Lowest levels were measured in peripheral nerve. Measurements of total tissue thiamin content provided no explanation for the selective vulnerability of the heart and peripheral nerve to thiamin deficiency. Total rat brain thiamin content was 3.21 μg/g wet weight, of which 4% was free thiamin, 11% thiamin monophosphate (TMP), 79% TDP, and 5% TTP. Similar ratios of phosphorylated thiamin to thiamin content were found in heart, kidney, and liver (42).

Studies of thiamin deficiency in rats showed a rapid fall in total thiamin levels in liver and heart after only 10 days of dietary thiamin restriction with only a minimal drop in brain levels (43). Studies of regional total thiamin levels in rat brain revealed few differences between the areas studied except for the lower levels of the thalamus, which averaged 9.2 μg/g dry weight, and the higher levels in the cerebellar vermis, average of 21.1 μg/g (41). Measurement of thiamin esters in cerebellum, pons, midbrain, and cerebral cortex of thiamin-deficient rats showed depletion of total thiamin levels in all areas tested in asymptomatic animals; the levels fell to a mean value of 41% of normal in symptomatic animals after 8 weeks of thiamin deprivation. Measurements of individual esters showed that most of the fall was accounted for by a loss of TDP. The greatest falls in TDP levels were found in pons and midbrain—two of the areas most susceptible to the lesions of thiamin deficiency (43).

A more recent study of rat brain and sciatic nerve thiamin turnover found total thiamin turnover times to be shortest in cerebellum and longest in brain stem (33). TDP turnover was short in all tissues studied, varying betwen 5.2 and 9.0 hr. The authors speculate this may be due to rapid dephosphorylation and rephosphorylation of TDP. Brain thiamin measurements in pediatric and adult disease control brain tissue were compared to those made in patients dying from subacute necrotizing encephalomyelopathy (SNE) (45). A marked difference was found between pediatric and adult controls: pediatric total brain thiamin was 0.69 ± 0.49 SD μg/g, while adult brain contained 2.22 μg/g ± 1.46 SD. Regional variations in total thiamin level were noted, with the mammillary bodies containing between 4 and 6 times the total thiamin content of other brain areas. TDP levels were not measured but TTP was found to be similar in both adult and pediatric control brains (average of 11%). TTP was reduced to 5% of total thiamin in SNE brain tissue.

Absorption

Thiamin is absorbed by the small intestine through at least two mechanisms (46). An active sodium-dependent ouabain-sensitive transport mechanism predominates at low concentrations, whereas at higher concentrations passive diffusion occurs (47). The kinetics of intestinal absorption in humans fits a Michaelis Menton model with a V_{max} of 8.3 ± 2.4 mg (48). Earlier studies suggested a maximum absorption of 4.8 mg following a 20-mg oral dose of thiamin in normal human controls (49). In humans there is little increase in urinary thiamin excretion when oral doses greater than 2.5 mg are administered (50), which suggests that passive absorption is not significant in the human. It follows that parenteral administration or the use of a lipid-soluble derivative such as thiamin tetrahydrofurfuryl disulfide given orally is necessary to achieve high systemic thiamin levels. A clue to the interaction of malnutrition in thiamin deficiency was provided by the observation that malnourished alcoholics have a decreased absorption V_{max} that increases after correction of protein-calorie malnutrition (49). Folate deficiency in rats decreases duodenal and jejunal thiamin uptake at low but not at high thiamin dosages (51).

Blood-Brain Barrier

Thiamin transport across the blood-brain barrier, like that across the gut, involves two different mechanisms, (52, 53). The saturable mechanism at the blood-brain barrier, however, differs from the energy-dependent mechanism described in the gut (46, 47) and from the active transport systems described in cerebral cortex cells (54, 55), which may be dependent upon membrane-bound phosphatases (56, 57). Studies in rats show that the thiamin analogues—pyrithiamin, thiamin disulfide, acetyl thiamin, and amprolinium—

selectively inhibit the blood-brain barrier saturable, carrier-mediated, transport mechanism. Pyrithiamin minimally inhibits the second nonsaturable transport mechanism. These data and the polarity of the thiamin molecule led to speculation that a transport molecule is also involved in the nonsaturable mechanism (58). Oxythiamin has no effect on blood-brain transport. Only minor regional differences were noted when thiamin and TMP uptake into rat brain were studied (53). A saturable mechanism (mean K_m 2.2 nmol/ml, mean V_{max} 7.3 nmol/g/hr) accounted for 95% of cerebellar and 91% of cerebral cortex uptake at physiological plasma thiamin concentrations. TMP transport rates were five to ten times lower than those of thiamin (53). Thiamin uptake rates were ten times the maximal rate of loss of thiamin from the brain (52).

Antithiamins

A number of naturally occurring compounds have antithiamin activity. These agents may affect both animals and humans. Population studies in northeastern Thailand found evidence of thiamin deficiency in red cells (25% showed significant changes in TDP effect) in spite of a daily thiamin intake exceeding the RDA (59). Fermented tea leaves, tea leaf extracts, and betel nuts were found to contain antithiamin factors. Removal of these foods improved the thiamin status of a number of study groups. Polyphenolic compounds in these foods, including tannic acid, seem responsible for the antithiamin activity and appear to act by oxidizing thiamin to thiamin disulfide. Ascorbic acid was protective. Thiaminase in raw fish was another contributing factor accounting for thiamin deficiency in this population; boiling the fish removed the effect (59). Urinary thiamin levels were reduced in human volunteers given coffee to drink. This was attributed to chlorogenic acid and caffeine (60). Two thiamin cleavage enzymes have been isolated from various natural sources (61). Thiaminase I, found in shellfish, raw fish, ferns, and a number of microorganisms, cleaves thiamin by displacing the methylene group in the pyrimidine moity. Thiaminase I also cleaves TDP. Thiaminase II is found in microorganisms and acts more simply by cleaving thiamin into its thiazole and pyrimidine moities.

Various naturally occurring diseases of the central nervous system (CNS) in animals have been shown to be due to thiaminases. Cerebrocortical necrosis in cattle and the related disorder polioencephalomalacia in sheep are caused by thiamin deficiency secondary to thiaminase activity in the gut lumen (62–64). Bilateral necrosis of cerebral cortex, lateral geniculate bodies, and posterior midbrain are the gross neuropathological findings in this disease (65). Chastek paralysis, a thiamin-deficiency polyneuropathy found in foxes fed on raw carp and in mink fed on uncooked fish, is caused by thiaminase. Bracken fern *(Teridium aquilinum)* contains thiaminase I and induces thiamin deficiency in rats fed a complete diet with fern powder added (66). Horses and pigs have also developed bracken poisoning.

A number of synthetic thiamin analogues have been developed. Amprolinium is a thiamin transport protein inhibitor used as an anticoccidal drug, predominantly in chickens. Amprolinium produces polioencephalomalacia in ruminants (67). Analogues containing a hydroxyethyl group similar to thiamin include pyrithiamin and oxythiamin. Because the production of the CNS lesions in experimental thiamin deficiency requires prolonged thiamin deprivation (8 weeks in rats), inhibitory analogues, commonly pyrithiamin, are often used in animal models. A number of differences have been demonstrated between the action of these various analogue inhibitors in brain (68, 69). Oxythiamin does not enter the brain, although it is capable, along with pyrithiamin, of inhibiting thiamin diphosphokinase (68). Oxythiamin-treated animals are markedly different from animals treated with either thiamin deprivation alone or pyrithiamin. Weight loss, anorexia, cardiac and adrenal enlargement, and elevated blood pyruvate levels are marked with oxythiamin treatment. Neurological effects, however, are not seen. CNS effects (lethargy, ataxia, and seizures) are more easily produced with pyrithiamin than with thiamin deficiency alone. The inhibitory effects of pyrithiamine on thiamin uptake at the blood-brain barrier probably account for some of this difference.

Pyrithiamin treatment and thiamin deprivation lower total brain thiamin, TDP, and TTP levels; oxythiamin has no effect (70). Brain acetylcholine levels were between 60 and 80% in oxythiamin- and thiamin-deficient animals. Less marked changes were seen in pyrithiamin-treated animals, who had more neurological symptoms (68, 71). Pyrithiamin treatment differs from thiamin deprivation alone in levels of brain neurotransmitters (72) and in its effects on pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (73, 74).

Evaluation of Deficiency

It is well established that measurements of urinary thiamin reflect intake but do not prove a subclinical deficiency. Over 20 metabolites of thiamin have been identified in urine. However, the pattern of urinary metabolites gives little information about thiamin status. The demonstration by Brin et al (75) of transketolase activity in erythrocytes and the stimulation effect produced by addition of TDP forms the basis for a sensitive test of the biochemical effects of thiamin deficiency. Transketolase is responsible for two reactions in the pentose phosphate pathway. The original assay for which most normative data is available measured the production of fructose-6-phosphate (75–77). A micromethod of measuring sedoheptulose-7-phosphate can also be used to measure TDP effect and detect subclinical or biochemical deficiency (78, 79).

The currently available assay procedures have been reviewed (80). One problem with measurements of transketolase activity and TDP effect is the dependence on kinetically normal transketolase enzyme. There is evidence

that in some patients with liver disease, the TDP effect cannot be demonstrated (36). More recently six different red cell transketolase isoenzymes have been identified (81). Levels of TDP effect necessary for identification of marginal and severe deficiency have been determined (12, 36, 76), although normative data should be established for each assay (12).

Thiamin condition	TDP effect
Normal	0–15%
Marginally deficient	15–24%
Severely deficient	25+%

In one recent study levels of TDP effect as high as 35.4% were found in subclinical deficiency (83).

Blood pyruvate and α-ketoglutarate levels are both elevated in patients with clinical manifestations of deficiency, but in asymptomatic patients only a small rise in α-ketoglutarate is seen. In patients with subclinical deficiency, pyruvate is generally elevated but difficulties in collection and stabilization of this ketoacid make interpretation uncertain (84). Glucose loading produces an elevation of pyruvate in asymptomatic patients but such loading is dangerous in patients with the clinical manifestations of deficiency (85).

THIAMIN IN CENTRAL NERVOUS SYSTEM FUNCTION

Energy Metabolism

Glucose is the major substrate for brain energy metabolism in nonfasted animals. However, in the fasted state 60% or more of human brain energy requirement can be supplied by ketone bodies (86, 87). Lactate, glycerol, amino acids, and free fatty acids can act as brain substrates. There is considerable species variability. Much of the metabolism of substrates other glucose and ketones is thought to occur largely in the glial cell pool (87). Activities of the α-ketoglutarate dehydrogenase complex and the pyruvate dehydrogenase complex in brain mitochondrial preparations are lower than other citric acid cycle enzymes, which suggests a rate-limiting role for these two thiamin-dependent enzyme complexes (88–93). Thus, derangement of oxidative metabolism of glucose and associated lactic acid accumulation will occur with a significant decrease in pyruvate dehydrogenase activity, and the oxidative metabolism of other brain substrates will be inhibited by a significant decrease in α-ketoglutarate dehydrogenase activity. An impairment of oxidative phosphorylation utilizing pyruvate, α-ketoglutarate, and succinate as substrates was demonstrated in rat brain mitochondria from pyrithiamin-treated animals (93).

Recent evidence links energy deficiency to the early morphological brain lesions observed in thiamin deficiency. Edematous changes in glia (94, 95) and hypertrophy and proliferation of membranes with periodically banded tubular structures in nerve terminals and axons (96) are the early ultrastructural changes seen in the rat lateral vestibular nucleus. Decreased ATP and phosphocreatine levels, together with an increase in lactate, were seen in this structure in pyrithiamin-treated rats (97). Studies in a rat pyrithiamin model using 5-kW microwave irradiation for 1.5 seconds to inactivate brain enzymes demonstrated a 10.5% decrease in ATP levels in the lower brain stem. Phosphocreatinine levels were lowered to 70 and 75% of control values in diencephalon and lower brain stem, respectively. These changes in phosphorcreatinine preceded the development of symptoms and histological lesions (95). Earlier studies in thiamin-deficient rats used slower brain fixation techniques by freezing the tissue in dry ice and acteone (98) or in liquid nitrogen (99). Changes in high-energy metabolites were not observed in these studies.

These early changes in phosphocreatinine led to speculation that the early astrocytic edematous changes observed in thiamin deficiency may be due to a low-energy state that decreased activity of the Na-K-ATPase, which in turn impaired ion transport at cell membranes. Regional pH was lowered in the inferior colliculus, medial dorsal nucleus of the thalamus, vestibular nucleus, and mammillary bodies in an autoradiographic study in pyrithiamin-treated rats (100). Local lactic acid accumulation is thought to contribute to the structural damage induced by hypoxia (101) and may contribute to the brain lesions in thiamin deficiency (100).

Values of pyruvate dehydrogenase complex activity in the brain have varied with differing assay procedures. Arylamine acetyltransferase assay methods generally produce higher values of activity than [1-^{14}C] pyruvate decarboxylation assays (73, 92). However, using intact brain mitochondria the [1-^{14}C] pyruvate decarboxylation assay can produce measures of activity similar to arylamine acetyltransferase measurements (90, 102). The fact that these higher levels of brain pyruvate dehydrogenase complex activity approximate those of measured maximal glucose flux in brain suggests that there is little excess enzyme available (103). Early studies found pyruvate decarboxylation activity unchanged in crude brain homogenates from thiamin-deficient animals. Brain stem activity was lower at 80% of control values, but pyruvate decarboxylation was more severely reduced in heart, liver, and kidney homogenates, averaging less than 50% of control (105). Later studies with a similar decarboxylation assay system in thiamin-deficient rats essentially confirmed these results (98, 106).

Using the arylamine acetyltransferase system, researchers found pyruvate dehydrogenase complex activity to be reduced by 14% in pons, 16% in

midbrain, and 33% in the lateral vestibular nucleus of symptomatic thiamin-deficient rats, but it was unchanged in the seven other areas studied. No regional changes in brain pyruvate dehydrogenase complex activity were observed in symptomatic pyrithiamin-treated rats (73). Work by others supports these findings (91). Studies in brain mitochondria from pyrithiamin-treated rats using a [1-^{14}C]pyruvate decarboxylation assay found decreased brain pyruvate dehydrogenase complex activity (93). Increases of up to 16-fold in α-ketoglutarate levels, with more modest levels of elevation of pyruvate, were found in the vestibular nucleus in a pyrithiamin mouse model (107). Studies in pyrithiamin-treated and thiamin-deficient rats demonstrated citric acid cycle impairment with α-ketoglutarate accumulation (68). Regional marked decreases in α-ketoglutarate dehydrogenase complex activity in pyrithiamin-treated rats with less marked reductions in thiamin-deficient animals have recently been reported (74). In these studies α-ketoglutarate dehydrogenase activity was reduced to 70% of control in the lateral vestibular nucleus and hypothalamus of symptomatic thiamin-deficient rats. However, in pyrithiamin-treated animals, more profound reductions of α-ketoglutarate dehydrogenase activity were seen in all brain areas, ranging from a reduction of 31.2% of control in the midbrain to 75.8% in the hippocampus. Even presymptomatic pyrithiamin rats showed significant decreases in enzyme activity. These changes in α-ketoglutarate dehydrogenase complex activity were completely reversed with thiamin (74). Other studies confirm both a decrease in brain α-ketoglutarate dehydrogenase activity and a return to normal activity following thiamin treatment in pyrithiamin-treated rats (91).

Studies to date suggest that impairment of the α-ketoglutarate dehydrogenase complex is more important in the pyrithiamin model than in thiamin deficiency. However, impairment of the citric acid cycle through decreased α-ketoglutarate dehydrogenase activity may play a major role in the production of energy impairment and the morphological changes in thiamin deficiency.

Pentose Phosphate Pathway

Although all enzymes in the pentose phosphate pathway are present in brain, the flux through this pathway in adult animals is only a few percent of the glycolytic flux (87, 108–110). In developing brain, however, the pentose phosphate pathway may metabolize as much as 50% of glucose carbon (87). Greater activity of the pentose phosphate pathway in younger animals is to be expected if its major role is the production of NADPH for biosynthetic functions. In addition to maintaining the NADPH redox state, the pentose phosphate pathway generates ribose phosphate for nucleic acid synthesis and reduced glutathione, which may act as a free-radical scavenger and is thought to maintain sulfhydryl groups in a reduced state (111). The rate-limiting pentose phosphate cycle enzymes, glucose-6-phosphate dehydrogenase and

6-phosphogluconate dehydrogenase, were unaltered in thiamin-deficient and pyrithiamin-treated rats. Transketolase values were decreased by over 60% in both cortex and brain stem, but flux through the pentose phosphate pathway was unaltered (112).

In mouse brain homogenates disrupted with triton X-100, transketolase was found to be tightly but not covalently bound to TDP. A mean activity of 5.7 ± 0.6 SE nmol/min/mg whole-brain protein was found (113). Earlier studies of brain homogenates without detergent treatment had found brain transketolase activity 20 to 50% of these values (98, 105, 112).

The early and marked fall in brain transketolase activity noted in many studies of thiamin deficiency was not related to the appearance of symptoms in experimental animals (98, 105). Furthermore, although thiamin supplementation rapidly reverses the symptoms and depression of brain pyruvate dehydrogenase and α-ketoglutarate dehydrogenase activities, blood and brain levels of transketolase do not return to normal (73, 74, 78, 98, 105). This suggests that thiamin deficiency affects the levels of the transketolase apoenzyme, which are not easily reversible by thiamin treatment.

Although in animal experiments transketolase does not seem related to the effects of thiamin deficiency, recent data suggest that transketolase may play a role in the development of the Wernicke-Korsakoff syndrome. Possible mechanisms have been reviewed (114). Initial reports of an altered transketolase K_m in Wernicke-Korsakoff patients (115) were not confirmed by other workers (81). However, a highly significant association was identified between Wernicke-Korsakoff syndrome and one of six transketolase isoenzymes demonstrated by isoelectric focusing (81). A faster-than-control decline in total erythroctye transketolase and transketolase-plus-TDP activity was found in Wernicke-Korsakoff patients after cessation of thiamin treatment (116). This may indicate a faster rate of degradation of transketolase in Wernicke-Korsakoff patients. Genetic abnormalities in red cell transketolase, suggested to underlie a predisposition to Wernicke-Korsakoff syndrome, may represent a damaged form of the enzyme (114). Increased amounts of the low-molecular-weight erythrocyte TDP transketolase variant were found in one patient during an acute alcoholic crisis, and levels of this variant reverted to normal two months after treatment (114). The suggestion of a genetic predisposition to Wernicke-Korsakoff disease, although attractive, remains unproven (114). While transketolase variants are found in Wernicke-Korsakoff patients, no proof for a transketolase role in the production of the brain lesions of thiamin deficiency currently exists.

Neurotransmitters

Alterations in a number of CNS neurotransmitter systems have been reported in thiamin deficiency, which suggests that thiamin is important for normal neurotransmitter function. Acetylcholine, gamma-aminobutyrate (GABA),

glutamate, and aspartate are produced primarily through the oxidative metabolism of glucose (117).

In most studies of brain acetylcholine levels in thiamin-deficient and thiamin-antagonist-treated rats, precautions to prevent the hydrolysis of acetylcholine postmortem have not been taken. Some investigators report reduced levels of acetylcholine (71, 118, 119) whereas others found no change in acetylcholine brain levels in thiamin deficiency (120–122). Studies using focused microwave radiation to prevent acetylcholine loss found no change in regional brain acetylcholine levels in thiamin-deficient rats. However, acetylcholine utilization was reduced in cortex, midbrain, diencephalon, and brainstem (123). In rat brain homogenates [3-^{14}C]pyruvate conversion to acetylcholine was unaltered in thiamin-deficient and pyrithiamin-treated rats. While acetylcholine synthesis was reported to be reduced in thiamin deficiency, it was increased in pyrithiamin-treated rats (68). A quantitative receptor autoradiographic technique revealed a decrease in muscarinic receptor binding in the ventromedial thalamus, but increases in muscarinic receptors were found in other brain areas in both thiamin- and pyrithiamin-treated rats (124). In other studies, acetylcholine turnover was reduced in both thiamin deficiency and pyrithiamin-treated animals (125). There is a general consensus that choline acetyltransferase is unaltered in thiamin deficiency states (118, 119, 126). Thus, although levels of acetylcholine may be unchanged, central cholinergic mechanisms do appear to be depressed in both thiamin deficiency and pyrithiamin rat models. Decreased pyruvate dehydrogenase activity limiting acetyl coenzyme-A production may underlie these changes (117, 127).

Cerebral catecholamine changes have been reported in thiamin-deficient rats (128). Reduced levels of cerebrospinal fluid metabolites of norepinephrine, dopamine, and 5-hydroxytryptamine (5-HT) were found in patients with Wernicke-Korsakoff syndrome (129, 130). Clonidine, a putative α-noradrenergic agonist improved memory impairment in some patients (131). Other investigators have not confirmed central catecholamine changes in Wernicke-Korsakoff syndrome (132). Recently behavioral deficits in rats persisting after reversal of thiamin deficiency have been linked to significant reductions in norepinephrine content of cortex, hippocampus, and olfactory bulbs (133).

Levels of four amino acids with putative neurotransmitter functions are altered in thiamin deficiency. Glutamate and aspartate levels were decreased in thiamin-deficient rat brain, with the most marked changes observed in the cerebellum (72, 134). Similar reductions in cerebellar GABA and glutamine concentrations were found in several brain areas in thiamin-deficient rats, with marked changes in the cerebellum (72, 117, 134, 135). Pyrithiamin effects on neurotransmitter levels appear to differ from those of thiamin

deficiency alone (72, 137). Cerebellar and brain stem glutamate concentrations are unaffected by pyrithiamin treatment. Aspartate and GABA levels were decreased in cerebral cortex of pyrithiamin-treated rats, whereas glutamine levels were increased in some brain regions. In cerebellar synaptosomal preparations from thiamin-deficient rats, aspartate and glutamate high-affinity uptake was increased. No significant changes were seen in norepinephrine, choline, GABA, taurine, and glycine uptake (137). GABA-transaminase and glutamic acid decarboxylase activities were reduced in symptomatic pyrithiamin-treated rats in thalamus, cerebellum, and brain stem (126).

Changes in 5-HT mechanisms appear more marked than those of other neurotransmitter systems in thiamin deficiency. In studies of cerebellar synaptosomal preparations from symptomatic thiamin-deficient and pyrithiamin-deficient rats, a marked decrease in high-affinity 5-HT uptake was observed. Changes were not significant in synaptosomal preparations from other brain areas. However, in studies of pyrithiamin-treated rats, a tenfold increase in cerebellar 5-HT turnover was found, and marked increases were observed in other areas. The 5-HT metabolite, 5-hydroxyindole acetic acid (5-HIAA), was significantly increased in all brain areas studied, but tryptophan levels were unaltered (137). These findings are supported by autoradiographic evidence of decreased 5-HT uptake in indoleaminergic afferents of cerebellum in thiamin-deficient rats (138). However, other investigators using immunofluorescent techniques could not find any change in 5-HT nerve cell bodies, brain 5-HT concentration, or tryptophan hydroxylase activity in young thiamin-deficient rats (143). Some studies indicate that the brain stem lesions seen in monkeys following intermittent thiamin deficiency are located predominantly in areas containing indolaminergic neurons and their processes (139). Because these animals exhibited a pattern of memory loss similar to Wernicke-Korsakoff syndrome (140), it is proposed that their amnesia is caused by a loss of 5-HT-containing neurons. It has also been proposed that the ataxia and thermoregulatory changes accompanying thiamin deficiency may be due to 5-HT neurotransmitter changes (141, 142). CSF levels of 5-H1AA were reduced in patients with Wernicke-Korsakoff syndrome, which provides further support for a disturbance of 5-HT metabolism (144).

Role in Nerve Conduction

Thiamin is important in nerve conduction, although its exact role is not known. An excellent review was published in 1979 by Cooper & Pincus (145). Thiamin deficiency causes peripheral neuropathy with an axonopathy. Nonspecific changes of axonal degeneration include disordered neurofilaments, and accumulation of degenerated mitochondria, vesicles, and small myelin figures (146). Early studies demonstrated that thiamin is located in

nerve membranes and mitochondria, but cannot be demonstrated histochemically in the axoplasm (147). The axonal membrane rather than myelin is the likely site of much thiamin membrane binding (148). In brain, thiamin has been shown to be associated with synaptosomal membranes (149). A concentration gradient with highest thiamin levels proximally was demonstrated in rat sciatic nerve. Both antegrade and some retrograde axoplasmic thiamin transport was shown by accumulation of fluorescence proximal and distal to axonal ligation (150). Similar results have been reported in frog sciatic nerve, where both uptake and transport of ^{35}S-thiamin were blocked by the oxidative phosphorylation uncoupler, dinitrophenol, and by inhibition of protein synthesis by cycloheximide (151). Reduced conduction velocity and increased axonal protein transport was recently demonstrated in sural nerves of thiamin-deficient rats (158).

The original observations of thiamin release with excitation of peripheral nerves in a number of different species were made fifty years ago (152). This thiamin release appears to be due to hydrolysis of TDP and TTP (153). In isolated frog sciatic nerve fiber, a number of thiamin antimetabolites, including pyrithiamin, alter the nerve action potential. Changes include an increase in the excitation threshold, broadening of nodal action potential, and a decrease in the rate of rise of the nodal action potential attributed to interference with the sodium channel (153, 154). Tetrodotoxin triggers release of thiamin from nerve (155), apparently by displacing TTP, TDP, and TMP from the sodium channel protein (156). A number of other neuroactive compounds including local anesthetics, acetylcholine, and 5-HT cause release of thiamin from nerve and nerve membrane fragments (145, 157). The finding that thiamin phosphorylated derivatives are associated with the sodium channel protein led to the hypothesis that TTP may play a fundamental role in the control of sodium conductance at axonal membranes (156).

Biosynthetic Role

Transketolase levels are reduced by up to 85% in developing thiamin-deficient rats, which may mean that myelinogenesis is particularly affected by thiamin deficiency. Although malnutrition causes a marked depression of total brain lipids with regional depression of cerebroside and cholesterol, these effects were no different in pair-fed compared to thiamin-deficient animals. Ganglioside concentrations were elevated (159). A 50% reduction in protein synthesis as indicated by ^{14}C-valine incorporation was found in cortex, brain stem, cerebellum, and subcortical structures of thiamin-deficient rats compared to pair-fed controls. These effects seemed primarily due to thiamin deficiency, but also partly related to hypothermia and malnutrition (160). Brain and visceral DNA synthesis is severely impaired in thiamin-deficient rats, but DNA levels were reduced only in liver (161). Thiamin-

deficient C-6 glial cells were found to have a significant depression (to 13% of control) in fatty acid and cholesterol synthesis. These effects seemed to be due to a reduction in activity of fatty acyl synthetase, acetyl coenzyme A carboxylase, and 3-hydroxy-3-methylglutaryl-CoA reductase (162).

Animal Models of Thiamin Deficiency

In general, animal models of vitamin deficiency have been poor models of human disease, in which deficiencies are often multiple and combined with protein malnutrition. However, in the case of thiamin deficiency, animal studies have been pivotal in our understanding of the pathophysiology of CNS damage.

Three models of thiamin deficiency have been studied in detail in the rat (68). These models are dietary thiamin deficiency and the administration of either oxythiamin or pyrithiamin to thiamin-deficient rats. The pyrithiamin–thiamin deficiency model appears to be the most practical model of thiamin deficiency, although brain lesions are more extensive and the effects of pyrithiamin on thiamin transport, neurotransmitters, and inhibitory effects on thiamin diphosphokinase and α-ketoglutarate dehydrogenase complicate the use of this model. Animals treated with pyrithiamin become symptomatic with ataxia, lethargy, poor appetite, and seizures and will die within 20 days of treatment.

The neuroanatomical distribution of lesions in experimental and natural thiamin deficiency has been reviewed (141). No single site or morphological change is characterisitic of thiamin deficiency. Hemorrhages, however, are more frequently seen in acute deficiency states. Considerable interspecies variability has been observed in the sensitivity of different brain areas to thiamin deficiency. Recurrent or prolonged periods of deprivation produce more extensive lesions in all species (141). Magnesium deprivation causes more severe histological changes in thiamin-deficient rats (163). Pyrithiamin produces more extensive lesions than simple thiamin deficiency in all animal models. In pigeons and rodents, acute and chronic thiamin depletion lesions are seen in pontine tegmentum, medulla, and cerebellum, but after a single period of pyrithiamin treatment in rats, additional lesions were seen in the thalamus and mammillary bodies; the cerebellum was spared (164). In Beagle dogs lesions in cerebral and cerebellar cortex as well as vestibular nuclei were found after prolonged thiamin deficiency (165). Recurrent periods of pyrithiamin treatment in mice and rats produce more extensive lesions in hippocampus, inferior colliculus, globus pallidus, cerebellum, and cerebral cortex (166–168). Widespread and variable brain lesions were found in cats surviving six months after pyrithiamin treatment (169). Differences between the CNS lesions of human thiamin deficiency and experimental thiamin deficiency in Rhesus monkeys have been noted (139, 140). The prominent

human lesions in the mammillary body and dorsomedial nucleus of thalamus were rarely seen in Rhesus monkeys.

Autoradiographic studies with ^{14}C-2-deoxyglucose in thiamin-deficient rats demonstrated increased local cerebral glucose utilization in the fornix, pyramidal tract, and inferior internal capsule of symptomatic animals. Thiamin administration reversed the increase in glucose utilization seen in these structures in parallel with symptomatic improvement. Thiamin did not, however, reverse the decreased glucose utilization observed in thalami, auditory structures, and vestibular nuclei (170). With pyrithiamin treatment in rats, local cerebral glucose utilization gradually declined in brain areas over the first 11 days of treatment. This was followed by a brief increase in utilization in 18 central areas that, along with the development of symptoms, later had a fall in glucose utilization (171). A similar distribution of glucose utilization changes was observed after chronic thiamin deficiency alone. Later it was shown that increases in local cerebral glucose utilization after short-term deprivation were reversed with thiamin administration (172). These important studies suggest that accelerated glucose utilization, probably due to increased glycolysis (Crabtree effect), precedes the symptoms and histological damage of thiamin deficiency but, for a period of time, this "biochemical lesion" is reversible.

THIAMIN IN HUMAN DISEASES

Subclinical Deficiency

There is concern about persisting borderline thiamin nutritional status even in developed countries (12). It is unclear whether psychological and neurological manifestations may accompany thiamin deficiency in the subclinical range. In a study of 19 male medical students, subclinical deficiency, defined as a TDP effect above 14.2% and below 35.4% in association with a low urinary thiamin excretion (<27 μg thiamin/g creatine), was associated with no measurable effect, even when a number of objective and subjective tests were applied (83). Irritability, frequent headaches, and unusual fatigue were reported after 12 days of the thiamin restriction in ten older women aged 52 to 72 years. Over this time period urinary thiamin excretion fell to 16 μg per day. Urinary thiamin levels fell to this level more slowly in eight younger women aged 18–21 years, and no subjective psychological changes were reported. Although the younger women received slightly higher daily thiamin intakes, this study raises the possibility that older subjects are more suseptible to the effects of subclinical thiamin deficiency (173).

In a recent study of 172 unselected psychiatric patients admitted to an English psychiatric hospital, 30% had red blood cell transketolase measurements suggestive of thiamin deficiency (11). Schizophrenia and alcoholism

were the commonest diagnoses in the low-thiamin patients, but no physical signs of deficiency were detected and more subtle psychological changes cannot be reliably evaluated in this patient group. Irritability was observed along with mild cardiovascular signs in a study of subclinical thiamin deficiency in Japanese university students: 42 thiamin-deficient students were identified from routine physical examinations of 766 freshmen. An additional 93 cases were found from studies of 2754 chest X-rays (13).

Although several studies suggest that mild mental changes may accompany subclinical thiamin deficiency, the major risk of borderline nutritional status is the increased risk of beriberi if antithiamin agents are introduced into the diet or if illness restricts intake further.

Taken as a whole, the US population receives an adequate dietary thiamin intake derived mostly (48%) from grain products. However, 17% of the population had intakes less than 70% of the RDA and 27% had intakes less than recommended thiamin-to-calorie ratios (10).

Certain groups are more susceptible to thiamin deficiency. A recent extensive review of thiamin status in Australia points out that dietary surveys in selected population groups since 1957 revealed considerable numbers of subjects with thiamin intake below 0.4 mg thiamin per 1000 kcal (177). At-risk groups included children, teenagers, elderly people, "adults in stress" groups, alcoholics, and some Olympic athletes (178). Biochemical studies showed a TDP effect of up to 24% in 40 apparently healthy hospital staff. TDP effects greater than this (severely deficient range) were found in 19% of Australian blood donors and 20% of alcoholic inpatients (179). In one study, as many as 90% of US institutionalized elderly had below adequate levels of thiamin based on urinary measurements, 43% based on red cell TDP response, and 100% based on diet analysis (180). A more recent review of several surveys indicates that 5% of the US population over age 60 have impaired thiamin status (181).

Beriberi

In developing countries thiamin deficiency is usually due to diets of milled rice or consumption of thiaminase-containing foods (6, 8). Other antithiamin factors may play a role (59). In developed countries, alcoholism is the major cause of thiamin deficiency, although food faddism and iatrogenic causes such as parenteral nutrition (182), chronic hemodialysis, and peritoneal dialysis account for some cases.

Beriberi is an excellent example of a vitamin deficiency disease whose treatment was revolutionized by the introduction of the crystalline vitamin in 1935–1936. Accounts of this disease in Shanghai (40, 183) detail the spectrum of clinical presentation. In the Chinese population, beriberi affecting adolescents and adults of both sexes was mild or subacute in 90% of cases.

Onset in half of the cases was associated with a nonspecific bout of fever acting as a stress. Elevation in blood pyruvate up to four times normal was noted, along with tachycardia due to cardiovascular involvement. Peripheral neuropathy was present in most cases, as was peripheral edema.

The acute fulminating form of beriberi (Shoshin) with high output cardiac failure accounted for 5% of cases in Shanghai. Acute cardiac involvement in alcoholics (11, 184) and in the Japanese population where thiaminase present in raw fish and clams is an etiological factor (61) continues to pose a problem even today. The 5% of patients with dry beriberi in the Shanghai population proved resistant to dietary or thiamin treatment, which implies irreversible neuropathy in this chronic form. An account of persistent neuropathy in ex–prisoners of war confirms these early observations (185).

Infantile beriberi was a common cause of infant death in rice-eating populations as early as 1901 (186). Beriberi was identified as the cause of a disease of Japanese and Philippine children termed "breast milk intoxication." The similarity of the brain lesions to Wernicke encephalopathy was noted (187). Infantile beriberi remains a significant cause of death among rice-eating people in rural areas of developing countries, with a peak incidence between 1 and 5 months of age. The disease was shown to be due to low thiamin content in breast milk from deficient mothers (6, 7, 176).

Early symptoms of infantile beriberi include anorexia, vomiting, listlessness, and constipation. Meningismus (pseudomeningitic type) may occur. Aphonia due to vocal cord paralysis can be seen (aphonic form). Encephalopathy with irritability progressing to coma, loss of eye movements, nystagmus, ataxia, and seizures complete the similarity to Wernicke-Korsakoff syndrome and are common manifestations of the subacute presentation. An acute cardiac death may occur as in Shoshin beriberi precipitated by stress such as infection (6, 7, 40, 176). In developed countries, infantile beriberi is rarely seen and may be misdiagnosed as subacute necrotizing encephalomyelopathy (189).

It has been suggested that low calorie intake may account for the rarity of Wernicke-Korsakoff syndrome in the Chinese population with beriberi and conversely the high calorie intake of alcoholics encourages the development of brain lesions (190). Acute deficiency of thiamin may encourage development of the Wernicke-Korsakoff syndrome (21) and carbohydrate loading can be an important precipitant (191). The development of wet beriberi in both its acute and chronic forms is favored by high physical activity and high carbohydrate intake (8, 40) whereas malnutrition and low activity levels favor beriberi presenting in the dry form with predominant peripheral neuropathy (40, 176, 183). Pure forms of all types of beriberi are found, but most patients with the cerebral form display peripheral neuropathy and some cardiovascular abnormalities. The cardiac and neuropathic forms often coexist.

Wernicke-Korsakoff Syndrome

The most comprehensive clinical and pathological study of Wernicke-Korsakoff syndrome to date was provided by Victor et al (192). The linkage of Wernicke's syndrome and Korsakoff psychosis stems from several studies reported in the German literature from the early 20th century in which the neuropathological changes in Wernicke disease were found in most patients with Korsakoff's psychosis (192). More recent large neuropathological studies suggest that the distribution of the brain lesions in Wernicke disease and Korsakoff psychosis are similar (192–194).

The clinical features characterizing Wernicke encephalopathy include global confusional state, nystagmus, opthalmoplegia, ataxia, and polyneuropathy. The chronic Korsakoff stage is characterized by an anterograde amnesia. The characteristics of the memory loss in Wernicke-Korsakoff syndrome have been reviewed (195). The symptoms of ataxia and ophalmoplegia are probably due to cerebellar and brain stem lesions, whereas Korsakoff's amnesia may result from lesions in the dorsomedial nucleus of the thalamus in association with atrophy of the mammillary bodies (192), although those lesions were not found in a monkey model (140).

Neuropathologically, acute, subacute, and chronic changes can be identified in Wernicke-Korsakoff syndrome (192, 193). Chronic changes include atrophy of the cerebral cortex and cerebellar vermis. Ventriculomegaly may accompany this. Atrophy of the mammillary bodies is found in the majority of cases. Acute changes include periventricular necrosis and hemorrhage, particularly in the wall of the third and fourth ventricles. Microscopically, perivascular hemorrhage is characteristic of acute lesions in which capillary proliferation, gliosis, and some loss of neuronal elements are seen. Subacute lesions are characterized by marked gliosis and loss of neurons, axons, and myelin sheaths. In chronic lesions most of the parenchymal elements are lost, to be replaced by gliosis and spongiform changes.

Although much of the literature reports Wernicke disease and Korsakoff psychosis in alcoholics, both disorders have also been reported in situations where thiamin deficiency was common. In the prisoner-of-war study, Wernicke disease was seen more commonly, but some patients progressed to Korsakoff psychosis (21). The occurrence of Korsakoff psychosis and polyneuropathy with hyperemesis gravidarum was reviewed as early as 1914 (197). Wernicke's disease has been reported in other situations associated with vomiting and malnutrition including Hodgkin's lymphoma, carcinoma of the stomach, chronic gastritis, and gastric partitioning (192, 198). Recent reports highlight the role of iatrogenic factors in the production of both acute beriberi (182) and the Wernicke-Korsakoff syndrome (191). Patients are usually chronically malnourished and receive carbohydrate without adequate thiamin. The view that development of Korsakoff psychosis requires factors

in addition to thiamin deficiency has recently been summarized (177). Poor response of Korsakoff patients to thiamin, the rarity of reports of Korsakoff syndrome in the absence of alcoholism, the high incidence of malnutrition and weight loss in alcoholics manifesting Korsakoff psychosis, and the occasional report of Korsakoff psychosis without preceding Wernicke disease are arguments that support the suggestion that Korsakoff psychosis is not a pure manifestation of thiamin deficiency.

Although Wernicke encephalopathy is frequently seen in the alcoholic population, studies of its true incidence are rare. Wernicke-Korsakoff syndrome accounted for 3% of all alcoholic neurological disorders seen at the Boston City Hospital (192). In an Australian study 26 of 1100 alcoholics had documented episodes of Wernicke encephalopathy (199). Autopsy studies indicate a much higher incidence of Wernicke-Korsakoff syndrome than is detected clinically. In one study of 1539 postmortem examinations, 1.9% showed Wernicke lesions (192); 131 cases of Wernicke-Korsakoff syndrome were found in a study of 4677 autopsied brains (194). The classical triad of symptoms (ataxia, opthalmoplegia, global confusional state) were not seen in 15 new cases of classical Wernicke disease, nine of which were diagnosed at autopsy. Nystagmus was found in only two patients and ataxia in three (200). In a recent Australian study, 80% of 131 autopsy-proven cases were unsuspected clinically. Of 97 cases for which data were available, 71% had no eye signs and only 16% had the classical triad (201). The discrepancy between clinical identification and autopsy-proven prevalence of Wernicke-Korsakoff syndrome indicates that chronic brain changes may result from repeated subclinical episodes of Wernicke encephalopathy (201). The fortification of alcoholic beverages with thiamin is recommended in a recent review (202).

Subacute Necrotizing Encephalomyelopathy

Subacute necrotizing encephalomyelopathy (SNE, Leigh's syndrome) was first described in 1951 (203). A comprehensive review detailed the clinical features in 86 patients (204). In 52 of these cases, the age at disease onset was less than 12 months; only eight developed their symptoms after the age of 2 years. Although the course, symptoms, and signs are heterogeneous, hypoventilation and apnea, cranial neuropathies, and hypotonia were the most common clinical features. Neuropathological features were studied in eight cases (205). Principal sites affected were caudate nuclei, putamena, periaqueductal tissues, tegmentum, and structures in the floor of the fourth ventricle. Lesions were also found in the centrum semi-ovali, cerebellum, and both grey and white matter of the spinal cord. Histologically, lesions in Leigh's syndrome bear a close similarity to Wernicke-Korsakoff lesions. Neuronal and axonal loss with spongiform change is seen, together with

gliosis and capillary proliferation. The distribution of lesions shows some differences. Spinal cord is often involved in SNE and mammillary bodies only rarely (205). SNE appears to be an autosomal recessive disorder in most cases, although X-linked inheritance has been suggested in some.

The involvement of thiamin in the etiology of SNE was suggested by the neuropathological similarity to Wernicke-Korsakoff syndrome and by the elevated blood and CSF lactate and pyruvic acid levels found in most patients. Some patients have partially responded to a low-carbohydrate, high-fat diet. Low TTP levels were reported in various brain areas from SNE patients (45). A nondialyzable substance that inhibits the TDP-ATP phosphoryltransferase reaction is present in the urine in some, but not all, patients with SNE (206, 207). Several reports describe abnormalities of the pyruvate dehydrogenase complex in SNE (208, 209). Defective activation of the pyruvate dehydrogenase complex attributed to phosphatase deficiency has also been reported (210, 211). Other workers, however, were unable to demonstrate abnormal phosphatase in SNE (212). A recent report described the clinical features of 30 patients with deficiency of the first component of the pyruvate dehydrogenase complex (208). SNE was found at autopsy in three of these. Cytochrome C oxidase deficiency has been found in several patients (188, 213). Pyruvate carboxylase deficiency has also been identified in some SNE patients (214, 215). It seems that the heterogeneous clinical syndrome of SNE is the result of disordered brain oxidative metabolism. A number of discrete biochemical defects can produce a deficiency of pyruvate oxidation. The similarity of SNE lesions to thiamin deficiency may result from a common failure of energy metabolism. The role of TTP in SNE remains unclear.

Thiamin-Responsive Maple Syrup Urine Disease

Maple Syrup Urine Disease was first described in 1954 in four patients who developed opisthotonos, intermittent increase in muscle tone, and respiratory abnormalities within the first week of life. In this severe form of the disease, the patient deteriorates rapidly and generally dies before the first month (216). The defect lies in the branched-chain ketoacid dehydrogenase complex. Leucine, isoleucine, and valine accumulate along with their corresponding ketoacids. Milder forms of the disease occur and are characterized by a higher level of residual dehydrogenase activity. Levels of activity in thiamin-responsive patients ranged from 4 to 40% of controls (217). A 15-year followup on the first reported patient is available (218). In spite of a low-protein diet and an early response to thiamin, this patient had five episodes of metabolic decompensation. A study of the reconstituted branched-chain ketoacid dehydrogenase prepared from disrupted fibroblast cells showed an elevated K_m value to α-ketoisovalerate, but with added TDP the kinetics appeared normal (219). In vitro and in vivo evidence suggests that TDP exerts

a stabilizing effect on the enzyme complex (220, 221). Although a response is not always seen, even in intermittent forms (222), all patients with Maple Syrup Urine Disease should have a trial of thiamin therapy.

Thiamin Toxicity

Large doses of thiamin cause death in various animal species by depressing the respiratory center (223). The vitamin must be administered intravenously in doses ranging from 125 mg/kg in mice to 350 mg/kg in dogs. Large intravenous doses in animals can also produce neuromuscular blockade (224). Because of limited gut absorption, large oral doses in humans do not appear to be toxic. Fatal anaphylactic shock and toxic reactions, however, have occurred following large intravenous doses (225). High serum thiamin measurements were reported in postmortem blood samples from infants dying with sudden infant death syndrome (SIDS) compared to controls (226). A later study, however, showed that total thiamin levels and free nonphosphorylated thiamin were markedly elevated in blood and CSF obtained postmortem from SIDS patients, non-SIDS controls, and adult controls. One SIDS victim was reported with antemortem blood thiamin levels within the control range followed by a highly elevated level 12 hours after death (196). The suggestion that SIDS is related to thiamin deficiency remains unconfirmed (174). Although normal erythrocyte transketolase activity was found postmortem in SIDS cases (175), the rapid postmortem release of thiamin into blood seems to invalidate measurements of postmortem TDP effect (196). Similarly, findings of decreased thiamin diphosphate and triphosphate in distal axons of phrenic and recurrent laryngeal nerves in SIDS cases may be artifactual (104).

CONCLUSION

Thiamin was the first vitamin to be identified, but human deficiency disease remains a significant world health problem. The molecular mechanisms underlying thiamin action in the brain remain an active area of study. A role in nerve conduction is well documented although the underlying mechanism remains unclear. The earliest biochemical studies documented an abnormality in the oxidative metabolism of glucose, and recent evidence suggests that a disruption in energy metabolism may underlie many of the neurochemical changes and structural lesions of thiamin deficiency. Much remains to be discovered about the role of thiamin in the brain.

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