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Thiamine Deficiency and Associated Clinical Disorders

Humana Press

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This volume is dedicated to Sue, April, Elizabeth, and Andrew

Preface

The past 20 years have seen remarkable advances in neuroscience, neurology, imaging techniques, and diagnostic strategies. These advances have been successfully applied to many different diseases, including thiamine deficiency and associated clinical disorders. Syndromes such as beriberi, Wernicke's disease, Leigh's disease, African Seasonal Ataxia, and various inherited ataxias have all benefited from improved scientific approaches. It is timely therefore to examine new knowledge related to the effects of thiamine deficiency on organ systems and to specific thiamine-related clinical disorders.

The elucidation of the biochemistry of thiamine took many scientists many years to complete and is inextricably linked to the study of thiamine deficiency. Thiamine (vitamin B1) is a water-soluble compound which consists of a pyrimidine nucleus and a thiazole ring. A key derivative of thiamine is thiamine pyrophosphate (TPP), called cocarboxylase. This form is a coenzyme which participates in the decarboxylation of several essential intermediates involved in carbohydrate metabolism. ATP is involved in the transfer of phosphate to thiamine. The two key decarboxylation reactions are the decarboxylation of pyruvic acid and of alpha-ketoglutaric acid. The enzyme transketolase is another enzyme requiring TPP as coenzyme. This enzyme is important in the hexose monophosphate shunt. The dietary requirement for thiamine is based largely on the caloric intake, and primarily that of carbohydrates. Thiamine is widely distributed in plants and animal tissues and is found in high concentrations in yeast, beans, wheat germ, oats, ham, soy beans, etc.

Thiamine is a naturally occurring vitamin which has many functions in mammals. An early and frequently studied role of thiamine is its participation as coenzyme in enzymatic functions in energy metabolism. Clinical descriptions of thiamine-related disorders were published many years before the association of their effects to thiamine deficiency. For example, beriberi was first described in medical literature in 1642 by the Dutch physician Jacobus Bontius (some of his writings related to beriberi have been translated and appear in Appendix A). It was many years later before the association between thiamine and beriberi was discovered. The enormous interest in thiamine deficiency and the associated clinical disorders stems from the fact that these represent classical metabolic encephalopathies, and as such are reversible (treatable) when recognized early.

As stated above, thiamine plays an important role as coenzyme in reactions in the tricarboxylic acid cycle and in the hexose monophosphate shunt. Thiamine deficiency produces symptoms which are primarily localized to cardiac and nervous tissues. These symptoms can be rapidly reversed by thiamine administration, which led to the important concept of a biochemical lesion. The term "biochemical lesion" implies a lesion which occurs before morphological lesions, the presence of which may render the disorder less amenable to treatment and reversal.

Thiamine may also play a role in neurotransmission, as well as several other key biochemical pathways. Perturbation of these important metabolic events may also contribute to key neurochemical alterations, and their reversal in thiamine deficiency and associated clinical syndromes. The fact that frequently the symptoms associated with both pure thiamine deficiency and those in human disorders related to thiamine deficiency can be reversed (successfully treated) in early stages is promising. If the biochemical lesion can be recognized and corrected before the onset of less reversible structural changes, recovery and return to normal function is possible.

Clinical disorders shown to be directly related to thiamine deficiency include beriberi, Wernicke's disease, Leigh's disease, African Seasonal Ataxia (ASA), inherited ataxias, central pontine myelinolysis, and others.

Pure beriberi heart disease is rare in the United States; however, it is endemic in other parts of the world. The majority of cases of beriberi (85%) are subacute and mild. The remainder of cases may be more severe, and if not treated, death may ensue. Since beriberi is a complicated nutritional disorder, it is not necessarily a disorder of thiamine deficiency alone; however, treatment with thiamine usually reverses symptoms, sometimes in only hours. There is a long, rich history relating to beriberi, including the pioneering work of Christian Eijkman, which resulted in the awarding of a Nobel Prize (Dr. Eijkman's Nobel Prize speech is reproduced in Appendix C).

Wernicke's disease, first described in 1881 by Carl Wernicke, occurs frequently in the United States and is usually associated with chronic alcoholism. Like beriberi, Wernicke's disease is a complicated nutritional disorder, but in the early stages, many symptoms can be reversed by thiamine administration. Wernicke's disease may have a rapid onset, and neurological symptoms consist largely of confusion, ataxias, nystagmis, and ocular palsies. Other brain stem symptoms may include stupor and coma. Many Wernicke's patients may lapse into the more chronic disorder, Korsakoff's psychosis. In later stages, symptoms may be associated with structural alterations in specific brain sites. Classic studies by Dr. Maurice Victor and colleagues on Wernicke's disease were carried out over many years, and are the basis for many publications in this area.

Leigh's disease (subacute necrotizing encephalomyelopathy) is an uncommon disease with around 200 cases reported in the literature. This disorder seems to be genetically transmitted. In patients diagnosed with Leigh's disease, there was found in urine an inhibitory substance which blocked the formation of thiamine triphosphate (TTP). Low levels of TTP were found in tissues of Leigh's disease patients. This was postulated to be responsible for thiamine-deficiency-like symptoms seen in these patients. Leigh's disease usually begins to be symptomatic around postnatal

month 6–12 following normal development. There appears lethargy, nystagmus, ataxia, failure to thrive, stupor, coma, and death. The lesions in the brain are usually more widespread than those in Wernicke's disease, but similar. Thiamine levels in Leigh's disease patients' blood and tissue are normal, and thiamine administration does not ameliorate the condition. A test for the inhibitor did not provide a conclusive indication of the presence of Leigh's disease, there being significant false positives and false negatives. Recent studies have shifted away from the inhibitor and examined supposed Leigh's disease patients for genetic defects including those with cytochrome c oxidase deficiency. Leigh's disease may represent one variation of the inherited ataxias. These studies and their ramifications will be described in depth. There are many unanswered questions relating to this fascinating disorder.

African Seasonal Ataxia (ASA) is another interesting recently described clinical entity which presumably has a thiamine-related foundation. This syndrome has been recently described in people who live in Western Nigeria and is characterized by ataxia, tremors, and decreased levels of consciousness. These symptoms occur during the rainy season of July through October. ASA usually follows a large carbohydrate meal. At its peak incidence, ASA can account for well over 70% of hospital and clinic admissions.

Various hypotheses have been advanced to explain ASA; however, strong evidence supports a mechanism related to thiamine deficiency. There is a clinical triad of cerebellar ataxia, ocular disturbances, and encephalopathy usually seen in acute thiamine deficiency. Upon examination of the dietary intake of the low socioeconomic strata of patients, it was found that almost all had consumed significant amounts of roasted silkworm larvae *Anaphe venata*. The availability of these larvae in the marketplace corresponds to the rainy season. The larvae represent a valuable protein source for rainforest people.

The practice of entomophagy in low socioeconomic cultures is accepted. Protein sources are relatively scarce for these people, who subsist largely on carbohydrate-rich diets. Subsequently, it has been shown that there is a thiaminase present in the *Anaphe venata* larvae. During the rainy season, these larvae fall from specific trees, and are gathered and sold in markets. Subsequent consumption of larvae containing a thiaminase by people ordinarily eating carbohydrate-rich diets can explain the rapid onset of symptoms resembling those of thiamine deficiency. As a corollary to this recent description are earlier descriptions of similar outbreaks of thiamine deficiency. There was, for example, the outbreak of the so-called Chastek paralysis, a disorder of silver foxes on a fox ranch in Minnesota. These foxes were fed raw fish and within a few weeks developed ataxia, changes in consciousness, seizures, and death. Pathologically, brain lesions resembled those seen in thiamine deficiency. Subsequent work showed the presence of a thiaminase in the viscera of the raw fish, which had precipitated the disorder.

Inherited ataxias are a group of relatively rare neurological disorders genetically transmitted, which have as a common denominator ataxia and the possibility of successful thiamine treatment. These diverse, yet related ataxias include Refsum's disease and Friedreich's ataxia. This group of disorders has a defect in the enzyme pyruvate decarboxylase. Pathologically, these disorders show mitochondrial damage

in selective brain regions. Treatment regimes include thiamine and ketonic diet therapies.

The disorder called central pontine myelinolysis (CPM) represents one variant almost always associated with some other serious chronic disease. Over one half of the reported cases were associated with alcoholism and Wernicke's disease. As the name implies, the key feature is a pathological lesion consisting of a symmetric focus of demyelination localized at the center of the pons. Microscopically, there is demyelination of medulated fibers. A variation of CPM are cases which, in addition to the lesion in the pons, demonstrate demyelinating lesions in other brain regions including the thalamus, cerebellum, cerebral cortex amygdala, putamen, etc. In spite of the predominance of alcoholism and Wernicke's disease as a feature of CPM patients, there are many instances of CPM in serious disorders not associated with thiamine involvement. The relation between thiamine and CPM therefore remains somewhat unclear.

Marchiafava-Bignami disease is yet another relatively rare disorder, which has been associated with thiamine deficiency. This clinical entity has as its distinguishing characteristic, lesions in the corpus callosum. These lesions lead to specific signs and symptoms, which together with MRI can lead to early diagnosis. Before imaging advances, the diagnosis was almost always made at autopsy. As a result of rapid diagnosis, treatment regimes involving thiamine administration have evolved, and cases of Marchiafava–Bignami disease have been successfully treated.

In summary, thiamine deficiency and associated clinical disorders represent an intriguing area of both basic and clinical investigation. Modern imaging and diagnostic strategies have facilitated the rapid treatment, and potential reversal of these clinical disorders. The fusion of laboratory and clinical knowledge serve as an example of how research can translate to successful treatment. This book is designed to bring together cogent results from both basic and clinical investigation. These data will be of interest to neurologists, internists, nutritionists, biochemists, neurochemists, neuroscientists, and many others with interest in thiamine deficiency. Many questions regarding these clinical disorders as well as thiamine deficiency remain unanswered. We hope this book may serve to stimulate further investigations in these areas.

Acknowledgments

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This volume would not have been possible were it not for the dedicated participation of Ms. Cristina Gonzalez. She was tireless in searching out old and new references, preparing tables, and organizing figures and figure permissions. Ms. Vilmary Friederichs also participated in organizing the manuscript and gave advice in the preparation of the volume. My son Dr. Jeffrey McCandless, NASA Ames Research Center, Palo Alto, CA, helped in the design of many excellent figures and in improving others. Finally, Ms. Ann Avouris, editor at Springer Science and Business Media, was outstanding in answering questions, helping obtain permissions for use of previously published figures, and in general shepherding this manuscript through the publishing process.

Contents

Early Chemistry

The early chemistry of thiamine deficiency goes back many years. The purpose of this introductory chapter is to review some of the remarkable discoveries relating to thiamine, thiamine-dependent enzymes, the relation to a deficiency of thiamine, and results, which led to the awarding of a Nobel prize to Christiaan Eijkman in 1929 in physiology or medicine for his work involving thiamine.

Remember that the first medical description of beriberi was published in 1642 by Jacobus Bontius (see Appendix A for a translation). Christiaan Eijkman, a physician, was able to demonstrate 250 years later that birds that were fed a diet of polished rice gradually progressed to a point where they became moribund and died. Eijkman had earlier tried unsuccessfully to isolate infectious agents that might have been responsible for beriberi. Eijkman next demonstrated that if the birds were fed unpolished rice, their symptoms reversed. The reversal feature of what was thiamine deficiency (but not known by Eijkman) was demonstrated very early in the investigations of the neurological (polyneuritis) outcomes of feeding polished rice. Also, Eijkman observed that the symptoms seen in pigeons closely resembled those seen in human beriberi.

The following is a description from Eijkman quoted verbatim concerning the symptoms in birds:

> "The beginning of the disease is characterized by an unsteady gait which first of all manifests itself in walking about on the perch, as if the animal cannot squeeze its toes around it firmly enough, and must exert itself in order not to fall off. The disturbance in mobility soon increases in intensity and speed. The fowl no longer has the strength to climb up; because of weakness it holds its limbs spread apart and bent at the knee and ankle joints; when running it frequently collapses or falls over. Finally, it remains lying on its side and in its fruitless efforts the developing paralysis of the wing muscles also becomes noticeable. The paralysis of the body musculature rapidly progresses from below upward."
>
> "The involvement of the peripheral nerves is the most important feature that post-mortem investigations reveal to date. It involves both the sensory and motor portions, which occur focally in the nerve trunks, and produces the picture of non-inflammatory atrophic degeneration such as is observed after transection of a nerve in the distal portion of the divided fragment. However, definite changes in the spinal cord and spinal cord roots are also not lacking. These show, likewise, the appearance of degeneration and atrophy."

D.W. McCandless, *Thiamine Deficiency and Associated Clinical Disorders*,
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Some years later, Eijkman and Vorderman (Eijkman, C. and Vorderman, A., 1897) were able to show that it was the "polishing" of the rice that was causing the symptoms of polyneuritis that were prevalent in birds and in man suffering the disease beriberi. Later it was discovered that a very small amount of a substance called "vitamine" by Funk (1911) was the compound which was essential for normal health, and the substance whose absence was responsible for symptoms in birds and man. The final "e" of vitamine was dropped when it was learned that the substances were not amines.

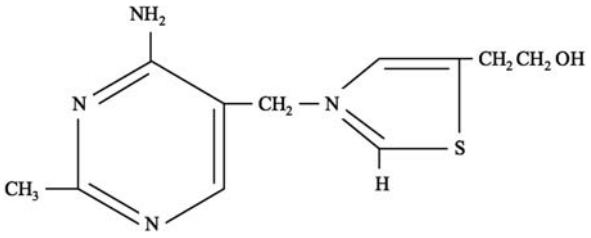
Early on Eijkman knew that polyneuritis was linked to the type of rice eaten. He believed that the symptoms were due to an infectious agent or toxin on the rice. Later it was shown that there was no toxin or infectious agent associated with the rice or its preparation.

Many investigators tried unsuccessfully to purify and isolate the mysterious vitamin until Jansen and Donath (1926) succeeded. It was a decade later before the vitamin was synthesized in the laboratory of R. R. Williams. Jensen and Donath sent a small amount of the compound to Eijkman who was able to show that as little as 2 μg was enough to protect pigeons from the deleterious effects of a diet of polished rice. Dr. Christiaan Eijkman received the Nobel Prize in Physiology or Medicine in 1929. His acceptance speech appears in Appendix C.[1]

Later in 1936, the structure (Williams, R., 1936), then the synthesis of the vitamin (Williams, R. and Cline, J., 1936) appeared as a communication to the editor of the Am. J. Chem. Soc. "Many laboratories were working on this problem, and later were able to confirm Williams" work.

The structure for thiamine put forth by Williams is as follows (Fig. 1):

Fig. 1



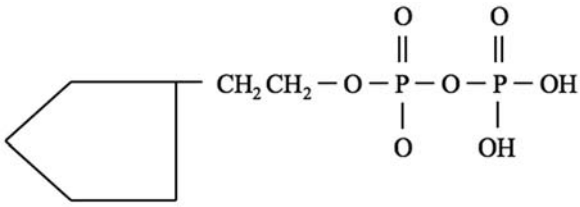
Thiamine

Using liquid ammonia, Williams was able to produce a free base of thiamine, $C_6H_{10}N_4$. A couple of months later, the synthesis of thiamine was briefly outlined by Williams. The synthesis included ammonia, $POCl_2$, and HBr. The structure and synthesis of thiamine was the culmination of many years of hard work.

[1] It is important to note that Frederick Gowland Hopkins shared the Nobel Prize in Physiology or Medicine with Eijkman in 1929. It was awarded to Hopkins for his work on vitamins and for his finding that muscle contraction leads to lactate accumulation.

The active form of thiamine is thiamine pyrophosphate or cocarboxylase. This active form has a structure as follows (Fig. 2):

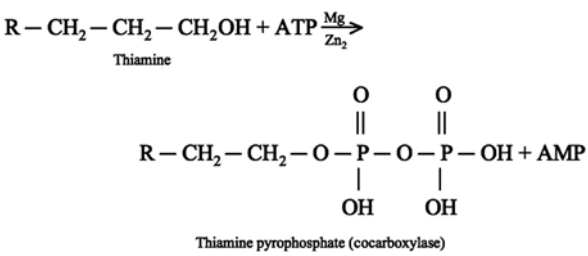
Fig. 2



Thiamine pyrophosphate (cocarboxylase)

One of the first to synthesize thiamine pyrophosphate was Weil-Malherbe (1940), who synthesized the compound by treating thiamine bromide with silver pyrophosphate. It was also shown about the same time that yeast, bacteria, and animal tissues could also generate thiamine pyrophosphate enzymatically (Fig. 3).

Fig. 3



Thiamine

Thiamine pyrophosphate (cocarboxylase)

Finally it was shown (Lohmann and Schuster, 1937) that thiamine pyrophosphate was the coenzyme "active" form of thiamine.

Three key enzymes in carbohydrate metabolism require thiamine in its phosphorylated form (thiamine pyrophosphate) in order to function properly. The two enzymes pyruvate and alpha-ketoglutarate dehydrogenases are of particular interest because of their pivotal role in metabolism. Pyruvate dehydrogenase is important in lipid synthesis, gluconeogenesis, and oxidation via the Krebs (TCA) cycle. The Krebs cycle produces much needed energy in the form of ATP. The third thiamine-requiring enzyme is transketolase, which is the rate limiting step in the hexose monophosphate shunt, which produces reducing power (NAD/NADH) and ribose.

The pyruvate dehydrogenase complex is regulated largely by product inhibition. ATP in micromolar concentrations is able to inhibit kidney pyruvate dehydrogenase (Reed, L., 1976). Similar compounds such as ADP, CTP, and GTP were not shown to be regulatory. Magnesium is also an important component of the enzyme complex, and when pyruvate dehydrogenase is phosphorylated, but inactivated, Mg can activate the enzyme.

The alpha-ketoglutarate dehydrogenase complex is large (2.7 million M.W.). It can be separated from other dehydrogenase complexes by fractionation (Koike, M. et al., 1976). Highly purified enzymes were analyzed for substrate specificity. Results showed that the pyruvate dehydrogenase enzyme complex has a rather wide range of substrate specificity in that it can decarboxylate other substrates besides pyruvate, including alpha-ketobutyrate, alpha-ketovalarate, and alpha-ketoceproate, among others. The enzyme alpha-ketoglutarate dehydrogenase was found to be specific for alpha-ketoglutarate. Both enzymes catalyze the first step of the two enzymes which decarboxylate pyruvate or alpha-ketoglutarate.

The possibility of important regulation of the pyruvate dehydrogenase enzyme is based on its critically important location in glycolysis, in lipid metabolism, and in the Krebs cycle. Studies have shown that the enzyme is inhibited by its products such as acetyl-CoA and NADH, and the enzyme reverts to normal activity in the presence of NAD and CoA. Other keto acids mentioned above were inhibitory for the pyruvate dehydrogenase enzyme complex. The binding of thiamine pyrophosphate to pyruvate dehydrogenase is loose, making the activity highly dependent on available thiamine pyrophosphate.

Examination of the effect of the three major methods of producing thiamine deficiency in mammals (diet only, diet plus oxythiamine, and diet plus pyrithiamine) shed some light on the enzymes that thiamine pyrophosphate serves as coenzyme (Gubler, C. and Johnson, L., 1967). Animals were Sprague-Dawley rats, and controls received 10 μg/100 g/day of thiamine supplementation. After sacrifice, tissue was separated, and pyruvate decarboxylase and alpha-ketoglutarate decarboxylase was measured in heart, liver, kidney, and brain. Results showed that pyrithiamine plus deficient diet decreased pyruvate decarboxylase in all tissues examined, while alpha ketoglutarate decarboxylase was decreased only in brain and kidney. Oxithiamine plus deficient diet caused a decrease in pyruvate decarboxylase in liver, heart, and kidney, while alpha-ketoglutarate was only reduced in the heart. Replacement of the carbohydrate diet (thiamine deficient) with a high-fat diet (which could have "fed" the system) had only a trivial effect.

Further studies showed that pyrithiamine was an inhibitor of the enzyme thiamine pyrophosphokinase, thus blocking the formation of thiamine pyrophosphate. Oxythiamine formed oxythiamine pyrophosphate, which was in turn an inhibitor of both pyruvate decarboxylase and alpha-ketoglutarate decarboxylase, as well as transketolase.

The structure of the actual enzymes pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase have been studied in part with electron microscopy (Reed, L., 1967).

The thiamine-requiring enzyme alpha-ketoglutarate dehydrogenase is actually composed of three enzymatic subunits: alpha-ketoglutarate decarboxylase, dihydrolipoyl transsuccinylase, and dihydrolipoyl dehydrogenase. When the three subunits are combined, they automatically form a large molecule, which resembles the structure of the alpha-ketoglutarate dehydrogenase.

The thiamine-requiring enzyme pyruvate dehydrogenase is also composed of three subunits: pyruvate decarboxylase, dihydrolipoyl transacetylase, and

dihydrolipoyl dehydrogenase. This composition is very similar to that of alpha-ketoglutarate dehydrogenase. These three components of pyruvate dehydrogenase form a three-dimensional configuration such that during enzyme functioning, the components are brought close enough to each other to function properly.

Electron microscopic studies of pyruvate dehydrogenase enzyme show a polyhedral form with a diameter of about 400 Å. Electron micrographs show that the transketolase moiety is located in the center of the polyhedron, and the other two complexes are located around the transacetylase. Electron micrographs also show the transacetylase moiety has eight subunits at the eight points of a cube. The transacetylase component is key to the overall three-dimensional form of the pyruvate dehydrogenase complex.

In terms of the biosynthesis of pyruvate dehydrogenase, studies indicate that the three-dimensional configuration of the complex is under direct genetic control. Little wonder that such a complex structural component, under genetic control, might have a variety of point mutations, any one of which could affect the functional capacity of either of these two thiamine-requiring enzyme complexes, pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase.

Many of the structural/three-dimensional studies have initially been performed in bacteria. Later studies in mammalian tissues such as heart and kidney show quite similar structural results. Electron microscopic examination show striking similarities to the enzyme in bacteria-based studies. Even down to basic detail, such as the position of the subunits on the transacetylase, components are similar to the bacteria counterpart.

A specific three-dimensional structure may enhance the ability of the complex to perform its duty. In addition, there may be a significant increase in the efficiency of the complex to dehydrogenate substrate. The close association of the three components should facilitate activity. This might be a sort of evolution in terms of efficiency of enzyme capacity to function.

Experimental evidence supports the concept that there is a protein which binds thiamine in bacteria (*Escherichia coli*) (Nose, Y. et al., 1976). Data examining the inhibition of thiamine binding to protein showed that classical thiamine analogs such as oxythiamine and pyrithiamine had little effect on binding, but that thiamine monophosphate, thiamine diphosphate, and thiamine triphosphate had over 50% inhibition. Chemical treatments such as iodine and bromosuccinimide had an over 50% inactivation of the binding of thiamine to protein.

The regulation of pyruvate dehydrogenase has been examined (Shen, L. et al., 1968). In this study, pyruvate dehydrogenase was isolated from *E. coli*. The enzyme was purified by protamine sulfate precipitation and elution (Koike, M. et al., 1976). Results showed that pyruvate dehydrogenase is inhibited by S-acetyl-CoA, and this inhibition is directed to the first reaction enzyme, a thiamine-requiring enzyme. Further regulating this first enzyme are nucleotide concentrations. Thus AMP, ADP, and ATP act to increase the rate of activity of the enzymes whether S-acetyl-CoA is present or not. While ATP in this preparation was stimulatory, in vivo it is probably inhibitory. It is important to consider only real physiological variables in vivo in attempting to assess regulation of pyruvate dehydrogenase.

Since the brain is not a gluconeogenic organ, the regulation of the thiamine-dependent enzyme pyruvate carboxylase by rat brain mitochondria has been investigated (Patel, M. and Tilghman, S., 1973). Rat brain mitochondria were isolated after decapitation and viability assured by measuring the P:O ratio and respiratory quotient. When all cofactors were present, the mitochondria fixed $H_{14}Co_3$ at a rate of 269 nmoles/30 min/mg protein. If pyruvate, $MgCl_2$, or ATP were left out of the mixture, fixation was nearly zero. Omission of Pi reduced decarboxylation by 65%. The combination of adenonucleotide and Pi data tend to show that the decarboxylation is regulated by intramitochondrial adenine nucleotide concentration. Except for citrate and isocitrate, the other dicarboxylate intermediates of the TCA cycle tended to decrease the decarboxylation of pyruvate as much as 70%.

The concentration of pyruvate also played a role in the regulation of mitochondrial decarboxylation. The role in regulation for the cofactor acetyl-CoA was not examined, but speculation is that it plays a role. The acetyl moiety of acetyl-CoA is vital for lipogenesis as well as the formation of acetylcholine. These are cytosolic processes, and the acetyl-CoA does not cross mitochondrial membranes. Data tend to show that citrate may act as a transporter to carry the acetyl moiety to the cytoplasm.

In another study (Tomita, I. et al., 1974), analogues of thiamine pyrophosphate were examined for their effects on pyruvate decarboxylase and transketolase, both thiamine-requiring enzymes. Results showed that both pyruvate decarboxylase and transketolase reconstitution was inhibited by thiazole pyrophosphate as well as by methyl and benzyl thiazole pyrophosphate.

The phosphorylated esters of 2-methyl and 2-benzyl thiazoles were inactive in combination with apopyruvate decarboxylase, or with apotransketolase. It appears to be the pyrimidine moiety of thiamine pyrophosphate which is important in binding studies, causing hydrogen bonding to take place. It is speculated that the pyrimidine moiety could act to tighten the binding of coenzyme by negating ionic repulsion.

Another study (Taylor, S. et al., 1975) describes the effects of the ATP/ADP ratios and other variables on the activity of pyruvate dehydrogenase. A rapid freezing method was developed to minimize any possible artifact change between active and inactive pyruvate dehydrogenase.

Results showed that the rapid freezing method was successful in extracting pyruvate dehydrogenase with little interconversion between the active and inactive forms of the enzyme. The investigators found a good correlation between the active form of pyruvate dehydrogenase and the intramitochondrial ATP/ADP ratio. Also noted was that pyruvate increased the active form of pyruvate dehydrogenase without any alteration in the ATP/ADP ratio. Octanoate acted to lower the active form of the enzyme, also without any significant change in the ATP/ADP ratio.

These data, taken together, lend strong support for the concept that the ATP/ADP ratio is an essential component for pyruvate dehydrogenase activity. This observation might be best explained by the fact that ADP is a competitive inhibitor of active pyruvate dehydrogenase kinase. The authors speculate that the ATP/ADP ratio in

mitochondria may also be a key regulator of the active form of the enzyme in intact cells.

The developmental pattern of pyruvate carboxylase has been studied in newborn rats because of its importance in gluconeogenesis (Chang, L., 1977). In these studies, animals from –5 days (premature) to 36 days after birth were utilized. Results showed that about 70% of pyruvate carboxylase activity was located in the mitochondrial fractions of liver. Twenty percent was found in the nuclear fraction. Only about 5% of adult levels of pyruvate carboxylase were found in fetal liver. The levels of enzyme remained low until birth, when activity levels rapidly rose, reaching a peak at about 5 days after birth. The actual rise after birth occurred after the first 8 hours, suggesting that the birth process in and of itself was not significant.

The developmental pattern of pyruvate carboxylase in vitro agrees with that seen previously in vivo (Snell, K., 1974). The author speculates that the rise in activity of the enzyme could be related to liver maturation. More likely is that the rise after birth is due to a sudden change from a high-carbohydrate diet (transplacental) to a diet of milk high in fat (nursing). This would spell a clear adaptation to changing dietary balance.

Early Thiamine Deficiency

Early thiamine deficiency studies were performed on pigeons because these birds were uniquely sensitive to polished rice. Symptoms frequently were evident by 21 days, which was as early as or earlier than any other animal model. This sensitivity also took the form of pronounced symptoms and certain death when not reversed with thiamine. The reversal feature was key in determining the significance of biochemical changes. If symptoms reversed with thiamine treatment along with key substrates such as decreased lactate, then this implied a role for lactate in the generation of the symptoms (Fig. 1).

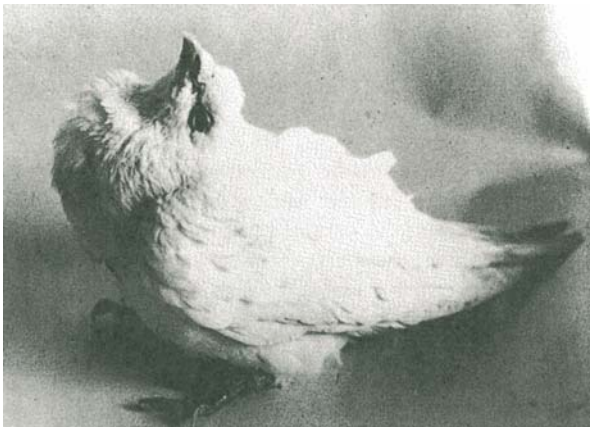


Fig. 1 Pigeon showing opisthotonic posturing due to thiamine deficiency. Reproduced from Yoshinori (1995) with permission from Springer

Several studies from about 1910 to 1916 were pivotal in developing concepts relating to vitamin physiology and biochemistry. For example, it was discovered that certain chemicals were necessary for continued health, and these were termed "vital amines," from which the word vitamin was coined. It was shown that adding carbohydrate to a vitamin-free diet brought about the development of symptoms more rapidly. This suggested that the carbohydrates require vitamins, and the addition of carbohydrates increases vitamin demand and consumption.

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Casimir Funk was instrumental in some of these early studies. In one report (Funk, C., 1911), he placed pigeons on several diets and the results were compared. The data showed that all pigeons placed on diets with high concentrations of carbohydrates developed hyperglycemia. Pigeons maintained on a vitamin-free diet also developed hyperglycemia. When hyperglycemic animals were treated with vitamins, the hyperglycemia diminished and liver glycogen levels increased. These studies show early correlations between carbohydrate metabolism and vitamins.

By the mid-1920 s several studies had been published, sometimes with conflicting data. Most investigators, however, were in agreement that animals deprived of vitamin B had significantly decreased oxygen consumption in various tissues. This was largely attributed to a reduction in the amount of activity of oxidizing enzymes. However, even in related measurements such as the respiratory quotient, there was disagreement. Hess and Messerle (1922) suggested that there was a similarity between the polished rice-fed beriberi pigeons and the result of sublethal doses of cyanide. He also speculated that the biochemical results in animals eating polished rice were manifest in a decrease in the amount or efficiency of oxidizing enzymes.

Given the somewhat controversial situation regarding various hypotheses that had been put forth to explain the relation of these findings to vitamin B metabolism, a rigorous study was published (Drummond, J. and Marrian, G., 1927). In one set of experiments, the authors compared the rates of reduction of methylene blue as a hydrogen acceptor to assess the oxidation/reduction status. Normal controls and beriberi tissues (pigeon breast muscle) were compared. Results did not confirm studies by Hess (Hess and Messerle, 1922). The Hess results showed that it took longer for the vitamin-deficient (beriberi) tissue to produce a reduction of methylene blue than in the case of normal non-deficient tissue. In the study by Drummond and Marrian, there was a small increase in time for the reduction of methylene blue, but it was not deemed significant.

Similarly when these investigators examined oxygen consumption in normal and beriberi tissues, results showed no real difference in breast muscle and only a small decrease in liver. These workers also documented the weight loss seen in the final days on vitamin B deficient diet. The authors conclude that on the basis of their findings, there is no evidence to support the view that vitamin B is essential for the functioning of the oxidative mechanism in tissues. The authors do point out that they cannot rule out the possibility that some small stimulation in oxidation might occur in intracellular locations. This could explain the small rise in temperature seen when animals deficient in vitamin B are administered extracts containing the vitamin.

In another study (Kinnersley, H. and Peters, R., 1930) published 2 years later, the relation between lactic acid in the brains of avitaminosis pigeons and normal control birds was described. Pigeons were stunned and the head was cut off and quickly frozen in liquid air. Frozen brain samples were extracted in 10% TCA, and following suitable preparation, lactic acid in the brain and in the blood was measured in normal pigeons and in opisthotonic pigeons. The vitamin-deficient pigeons were made opisthotonic by feeding them with polished rice. Results showed a much higher concentration of lactic acid in the brains of opisthotonic pigeons than in that of normal control birds.