

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.

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.

Worried that some artifact of lactic acid might have occurred due to the few seconds delay before the brain was frozen, the authors constructed a special guillotine. This device was described as “T” shaped with two blades at different levels. The lower blade decapitated the pigeon, while the second, higher blade split the skull. This effectively severs the blood supply to the brain, and the splitting of the skull facilitates more rapid freezing.

Results using the improved guillotine showed elevated lactic acid, as was seen earlier. The increased lactic acid was noted during the opisthotonic stage, not earlier, and the elevated lactic acid rapidly returned to normal when the animals were treated (reversed). Results also showed that stunning produced higher levels of lactic acid than did anesthesia with ether. The authors conclude that lactic acid levels seen in brain are more than those that could result from blood levels, thereby suggesting storage of carbohydrate in brain. The rapid reversal with vitamin B1 indicates no other food material or ions could have been deficient enough to produce the severe symptoms seen in these pigeons.

It is amazing to see that in 1925 investigators (Kinnersley and Peters) knew about the lability of brain metabolites. They designed and built a two-bladed guillotine to first cut off the head and then split the skull, rendering easier access to the brain for freezing. This worked to prevent or minimize artifacts after death. Interestingly, in the late 1960 s into the early 1970 s there was intense concern about the very real risk of changes occurring in brains of experimental animals following killing. In fact, devices were designed to minimize changes. These included freeze blowers, microwave ovens, and brain choppers which “fixed” or froze tissue in milliseconds, rendering the measurement of “true” levels of labile metabolites. One wonders if those investigators knew of Peters’ attempts along the same lines, which were done 50 years earlier.

In another study by Peters (Gavrilescu, N. and Peters, R., 1931), oxygen uptake was determined in pigeon brain regions during vitamin B deficiency induced symptoms. In this study, oxygen uptake was performed rapidly after killing, the elapsed time being 12–17 minutes. The guillotine developed and described above was used. Brain regions were isolated and measured separately. Regions sampled included cerebellum, cerebrum, optic lobes, and “lower parts.” The speed of tissue preparation was attributed in part to a new air-damped Sartorius balance. The Homer pigeons were fed in the standard way for producing severe symptoms.

Results showed the usual symptomology of opisthotonus, and death if not treated. Oxygen uptake was determined in these birds with head retraction or just before these symptoms were apparent. Oxygen uptake in symptomatic birds’ cerebellum was comparable to normal birds, but values in the cerebrum, optic lobes, and lower parts were decreased in symptomatic as compared to normal birds. The most significant decreased areas were the cerebrum and optic lobes. It was further shown that the oxygen uptake reverted to normal when the animals were “cured.”

The authors state that these oxygen uptake data, together with earlier published lactate data, show conclusively that the disorder in vitamin-deficient pigeons represents a biochemical defect that is central in origin, not peripheral. The rapid reversal of opisthotonus and decreased oxygen uptake with vitamin B treatment prove that

these features are due to vitamin B1 deficiency, not starvation. The fact that the cerebellum was unaltered as regards oxygen uptake serves as an internal control, arguing against changes in blood circulation in the affected pigeon brain. The authors state that they do not think some circulating toxin is involved.

It is interesting to note that even today (2009) many neurochemical studies are performed on whole brain, neglecting the brain's heterogeneity, whereas 80 years ago regional brain studies were being performed, and regional differences in neurochemical responses to perturbations were being described.

In a later study (Gavrilescu, N. and Peters, R., 1931) the issue of the possible local lack of vitamin B1 was addressed. Arguing in favor of a local change are data showing regional alterations in biochemical paradigms such as oxygen uptake, and the rapid (30 minutes) reversal of symptoms by injection of thiamine into the cranium. The counterargument was that some hormone or some other substance might be stimulated and/or released into the circulation by vitamin B1, thereby causing the reversal of biochemical changes and symptoms. The authors chose to examine oxygen uptake *in vitro* when vitamin was added to a brain preparation from symptomatic pigeons.

Animals used were pigeons in the final stages of vitamin B1 deficiency and showing the symptom of opisthotonus. Brain areas used were optic lobes, cerebrum, and lower brain parts. Tissue was collected as before, minced, and oxygen uptake measured as previously described. After equilibration, vitamin B1 was added to the mixture. Results showed that when B1 was added to optic lobe and to the lower parts, there was a clear increase in oxygen uptake by the homogenate. Various methods of inactivation of vitamin B1 were tried, and only when the vitamin was completely inactivated did the addition fail to increase oxygen uptake.

Earlier results indicated the cerebrum acted differently, and in these experiments the effect of vitamin addition had little effect on oxygen consumption. The authors conclude that whatever decrease in oxygen consumption is present in the cerebrum, it is not due to vitamin B1 decrease, as it is not reliably reversed by the addition of vitamin B1.

Adding vitamin B1 to a regional brain preparation seemed to rule out any stimulation *in vivo* from some other agent or hormone in vitamin-deficient pigeons. Similarly, these data correlate well with the lactate data gathered earlier. Collectively, the data on lactate and oxygen consumption indicate a local (regional) biochemical lesion, which correlates with an abnormal condition in the CNS. The authors conclude that vitamin B1 is directly involved with oxidative metabolism in lower brain parts in symptomatic pigeons.

In a later paper (Rydin, H., 1935), pigeons that were not in opisthotonus but rather were ataxic due to vitamin B1 deficiency were studied. The goal was to see if there was a change in oxygen uptake in regional brain parts when other symptoms were present.

As before, pigeons were made symptomatic by feeding them polished rice. Birds were on this diet for at least 30 days and were selected for the study based on the absence of opisthotonus and presence of leg weakness and ataxia. Animals were killed by decapitation and brains quickly removed and minced with a

spatula. Oxygen consumption was then measured in the cerebrum and in the lower parts of the symptomatic pigeons' brains.

Results showed that pigeons exhibiting leg weakness had a greater increase in oxygen uptake due to added vitamin B1 in the cerebrum. There was a negligible effect in the optic lobes and the rest of the brain. In previous work the optic lobes and the rest of the brain were affected during the opisthotonus phase of vitamin B1 deficiency.

In this study, as seen earlier, the decrease in oxygen uptake and symptoms correlated with vitamin B1 deficiency, not inanition. This study shows this even in the absence of opisthotonus, but with leg weakness, a decreased oxygen uptake was reversed by vitamin addition *in vitro*, again arguing against the stimulation of some other hormone or chemical release. Finally this study demonstrates that a different brain region – the cerebellum – appears to respond to leg weakness. This shows that the heterogeneity of the brain accounts for different responses, which correlate with different neurological symptoms. This study points out again that the leg weakness was central in origin and not manifest due to altered muscle biochemistry or peripheral nerve involvement.

In a subsequent paper the oxygen/pyruvate ratio and the pyruvate RQ in normal and vitamin-deficient pigeon brain (McGowan, 1937) were determined. The methodology was as previously described from the Peters' laboratory.

Results showed that pyruvate was incompletely oxidized, and it was likely that some intermediate was accumulating. Also it seemed that some pyruvate was completely oxidized, while some was metabolized along some other route. Data from another worker showed that some pyruvate was metabolized to lactic acid and CO₂. Assuming that two-thirds of pyruvate is completely oxidized, and one-third goes into the Krebs cycle, then the theoretical oxygen/pyruvate ratio is around 450 and the RQ is 1.29, and these are about the actual values found.

The experiments described above were carried out in pigeons suffering from vitamin B1 deficiency due to eating polished rice. Results showed that the oxygen/pyruvate ratio was significantly lower than that of normal pigeons. The author suggests that there may be two different routes for pyruvate, and absence of vitamin B1 may influence which route is utilized in vitamin B1 deficient birds.

In a neuropathological study, pigeons were made thiamine deficient by dietary means, then brains and peripheral nerves were examined in animals exhibiting opisthotonus, leg weakness, and in controls without symptoms (Swank, R., 1940). Experimental groups consisted of the following: (1) starved and thiamine deficient, (2) acutely thiamine deficient, (3) chronically thiamine deficient, and (4) starved on normal diet. Acute thiamine deficiency was produced by tube feeding a deficient diet until vomiting occurred. Then thiamine was administered along with the diet for 3–6 weeks. After that, symptoms (opisthotonus) occurred in 7–12 days if the thiamine supplementation was stopped. In the chronic group, after vomiting occurred, a small dose (7–15 µg) of thiamine was administered. In these birds, ataxia and leg weakness occurred between the 16th and the 30th day.

Results in the acutely thiamine-deficient group showed occasional degeneration in the sciatic nerve only. Lesions were also seen in the ventral funiculus of the

cervical spinal cord. The lesions were those of degenerating myelin sheaths. It was thought that there were more affected nerves, but that the chlorate-osmic acid staining method would not show them.

In the chronically thiamine-deficient group, pigeons developed leg weakness and ataxia, not opisthotonus. In addition most of these pigeons showed evidence of heart failure. Results showed that the greater and longer lasting symptoms correlated with more pronounced evidence of demyelination in peripheral nerves. In mildly symptomatic birds, the pathology was distal, and as the intensity of symptoms grew worse, the lesion moved centrally. The process of degeneration was Wallerian in appearance. Centrally, lesions start in the lateral funiculus and follow the spinocerebellar tract and can be traced into the cerebellum. This "ascent" was similar to that seen peripherally where lesions first appeared distally and moved centrally in increasingly severely symptomatic birds.

In birds "reversed" with thiamine treatment, the total number of myelinated nerve fibers in the sciatic and brachial nerves increased as the birds recovered. Complete recovery took 6–9 weeks in birds in which leg weakness had lasted for 7–10 days before instituting treatment. This is in contrast to the rapid recovery (hours) in rats reversed with thiamine therapy. Microscopically, dorsal ganglia continued to show chromatolysis until symptoms cleared. It seemed that complete regeneration of nerve fibers had to occur before symptoms disappeared.

The author suggests that the lesion associated with opisthotonus is biochemical in nature, and that explains the rapid reversal of symptoms when the symptom is opisthotonus. By contrast, the leg weakness appears to be a lower motor neuron type of paralysis, and nerve fibers in the sciatic nerve degenerate. Adding thiamine to the diet reverses the lesion and symptoms, but the symptom reversal is dependent upon reversal of the nerve lesion. Hence, this reversal time is lengthy. The large myelinated peripheral nerve fibers degenerated first in thiamine-deficient birds. In the CNS, the long ascending spinocerebellar fibers were affected early in the process. When an axis cylinder has been damaged for longer than 4 days, changes in the cell bodies consisting of chromatolysis occur. The chromatolysis remains after initiation of the thiamine reversal period and finally disappears after the myelin cylinder shows complete repair. In the CNS, damaged neurons did not regenerate. It is suggested that in early thiamine deficiency, CNS neurons are at first damaged only chemically. If thiamine is administered during this early period cell recovery is about 100%. Later, structural changes occur in the cell body, which disappear only if the axis cylinder makes a full recovery.

Opisthotonus is a manifestation of decerebration. Leg weakness, a more chronic disorder, is due to degeneration of nerves such as the sciatic nerve. Reversal is possible given thiamine treatment and time.

The blood lactate/pyruvate relation in thiamine-deficient pigeons was investigated (Stoltz, E. and Bessey, O., 1942). Pigeons were fed the usual polished rice diet and blood was collected from a wing vein. Birds were on a thiamine-deficient diet for 9 days. At this point, blood sampling revealed increases in pyruvate, at a time when the pigeons were still asymptomatic. The birds were then allowed 5 more days of thiamine-deficient diet, at which time some pigeons were exhibiting mild

opisthotonus. This was the time at which the pyruvate values were greatest. Treatment of symptomatic pigeons with thiamine reversed these elevated pyruvate levels. This study demonstrated the usefulness of blood pyruvate/lactate measurements as a way to evaluate thiamine deficiency even before the onset of symptoms.

In an early study in rats (Harper, H., 1942), the rate of absorption of glucose from the intestine was examined. Rats were placed on a thiamine-deficient diet and maintained in a way that allowed a "subacute" thiamine-deficient state to exist. At the time of killing, glucose was administered, and after 1–2 hours, the entire digestive tract was removed and analyzed for glucose. Results showed that there was a significant decrease (17%) in glucose absorption. This decrease in glucose absorption was not surprising in light of the decreased function in the GI tract. In another series of experiments, the author found decreased glycogen in the liver of thiamine-deficient rats 3–6 hours after feeding glucose. This finding could be attributed to lower absorption of glucose.

The effect of oxygen and thiamine pyrophosphate (cocarboxylase) on the formation of citrate and alpha-ketoglutarate has been studied (Coxon, R. and Peters, R., 1950). In this study, pigeons were used and treated by administering polished rice as the diet until opisthotonus developed. Animals were killed and brain homogenates were prepared. Both citrate and alpha-ketoglutarate were measured. Results showed that the addition of cocarboxylase and oxygen to pigeon brain extracts promoted the generation of citrate and alpha-ketoglutarate. In pigeon brain preparation from thiamine-deficient animals, pyruvate accumulates. Previously it had been suggested that some seven-carbon compound was the first compound formed in the decarboxylation of pyruvate. This seems not to be the case given that it is pyruvate which accumulates when citrate and alpha-ketoglutarate do not form in thiamine-deficient pigeon brain. Thus it is shown that citrate and alpha-ketoglutarate are formed in the presence of oxygen and cocarboxylase. Further, it is concluded that a two-carbon compound is formed when pyruvate is decarboxylated.

Based on earlier work showing that thiamine deficiency interferes with the degradation of pyruvate, the author of this paper (Liang, C., 1962) examined pyruvic acid changes in thiamine deficiency.

Albino rats were made thiamine deficient by feeding a diet of polished rice powder. Pyruvic acid and total keto acids were measured. Results showed that rats on a thiamine-deficient diet died after 10 weeks, and they demonstrated weight loss and ataxia.

Pyruvate and keto acids in brain, heart, liver, and muscle showed a steady rise after administration of the deficient diet. Glyoxylic acid was detected in all tissues tested, including brain.

A slight decrease was seen in the rise in pyruvic acid and keto acids between days 15 and 22, and the reason is unclear. The reason for the elevation in brain of glyoxylic acid also remains unclear, but may be related to the deamination of glycine and the amination of alpha-ketoglutarate to glutamate and glutamine. Glyoxylic acid is toxic. These data are in keeping with previous reports showing impairment in enzyme activity of pyruvate decarboxylase and of alpha-ketoglutarate decarboxylase in the brains of thiamine-deficient rats.

In a paper by Lofland et al. (1963), two different strains of pigeons were studied as to the effect of thiamine deficiency on thiamine-dependent enzymes. Results showed that the “snow racer” pigeon was more susceptible to thiamine deficiency than the “white carneau” breed. This was viewed as an indication of a possible genetic control of thiamine-dependent enzymes. The enzyme alpha-ketoglutarate decarboxylase seemed to show the most decreased activity among the three enzymes studied. Results also showed that transketolase decreased in blood, but remained about normal in brain. The activity of pyruvate oxidase was decreased, which seemed to correlate with the elevation in pyruvic acid. Overall these data are in keeping with previous data showing an alteration in the thiamine-requiring enzymes in carbohydrate metabolism (for further review, see Itokawa, 1995).

Thiamine Deficiency and Associated Clinical Disorders

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2009, XIV, 192 p. 44 illus., 11 illus. in color., Hardcover

ISBN: 978-1-60761-310-7

A product of Humana Press