Thiamine Triphosphate Deficiency in Subacute Necrotizing Encephalomyelopathy

Abstract. *Extracts of tissue fluids from a patient with subacute necrotizing encephalomyelopathy inhibit thiamine pyrophosphate–adenosine triphosphate phosphotransferase of rat brain. Brain tissue from the patient, in contrast to normal brain tissue, contained essentially no thiamine triphosphate, although thiamine and its other phosphate esters were present in normal concentrations. These findings suggest a relation between this disease and thiamine triphosphate.*

Subacute necrotizing encephalomyelopathy (SNE) is a degenerative disease of childhood which generally becomes symptomatic in the first year of life and causes death within 12 months. The absence of easily recognizable clinical features or helpful laboratory tests has made definite antemortem diagnosis difficult in cases in which no family history of the disorder was present. Pathologically, the disease resembles Wernicke's encephalopathy, and mainly for this reason it has been thought to result from a disorder of thiamine metabolism. The high familial incidence of this disease supports the contention that it is a genetic disease (*1*).

A child with SNE whose sister died of the disease 3 years ago was studied. Blood samples were assayed for activities of pyruvate dehydrogenase (E.C. 1.2.4.1), α-ketoglutarate dehydrogenase (E.C.1.2.4.2), and transketolase (E.C.2.2.1.1) [the three thiamine pyrophosphate (TPP) dependent enzymes], and the activities were well within the normal range. However, urine, spinal fluid, and deproteinized extracts of the patient's blood were found to inhibit thiamine pyrophosphate–adenosine triphosphate (TPP–ATP) phosphotransferase of rat brain, an enzyme system that catalyzes the synthesis of thiamine triphosphate (TTP) by a transphosphorylation reaction (*2*).

The enzyme used in this assay was prepared by homogenizing rat brain in 0.25*M* sucrose (nine volumes) containing 1 m*M* ethylenediaminetetraacetate and 10 m*M* glycylglycine buffer (*p*H 7.4). The nuclei were removed by centrifugation at 700*g* for 15 minutes and the mitochondria were then isolated by centrifugation at 7000*g* for 15 minutes. The mitochondrial suspension was washed three times with the sucrose medium before being suspended again in a small volume of sucrose. The phosphotransferase activity was assayed in two ways: (i) with TPP and ATP as substrates measuring the formation of adenosine diphosphate (ADP) spectrophotometrically by way of reactions with pyruvate kinase and lactate dehydrogenase (*3*); and (ii) with TTP and ADP as substrates measuring the formation of ATP spectrophotometrically by way of reactions with hexokinase and glucose-6-phosphate dehydrogenase (*4*). In a few experiments the enzyme was assayed using an electrophoretic and fluorometric procedure (*5*).

All urine specimens and several blood samples (both control and those of the patient with SNE) were treated electrolytically or by passage through a Sephadex G-25 column to remove the salt and eliminate artifactual inhibition before being assayed.

Regardless of which assay was used the results were the same and indicated that a factor (or factors) that inhibited this enzyme was present in the patient's blood, urine, and spinal fluid (Table 1). The factor has not yet been identified but some of its characteristics have been delineated. Boiling for 1 minute in 0.1*N* acid destroys the inhibitory effect of SNE urine, but boiling in alkali does not. The inhibitory effect was not lost by passage of the urine through columns of Dowex-1 or Dowex-50 resins nor was it lost by dialysis against distilled water for 5 hours. In the frozen state the inhibitory activity declines with time regardless of *p*H.

Samples of the patient's brain, kidney, and liver were removed 45 minutes after death and assayed for thiamine and its phosphate esters (*5*). No measurable amount of TTP could be found in the brain samples, in contrast to liver and kidney specimens (Table 2). Samples of brain, liver, and kidney obtained shortly after death from patients who died of a variety of diseases were also assayed for thiamine compounds and are referred to as "normal" in Table 2. The total thiamine content of the normal brains ranged from 62 to 261 μg/100 g, and that of the patient's brain was 40 μg/100 g.

The present finding that TPP–ATP phosphotransferase is inhibited by extracts of blood, urine, and spinal fluid from a patient with SNE suggests that the loss of activity of this enzyme may be causally related to the disease. Support for this contention comes from the finding of the virtual absence of TTP in the brain of the patient. The presence of TTP in kidney and liver of the patient suggests that the inhibitor acts only upon neural tissue. The fact that the blood of the patient's father had some inhibitory effect on this enzyme

Table 1. The inhibition of the TPP–ATP phosphotransferase of rat brain by blood and urine of a patient with subacute necrotizing encephalomyelopathy. To a cuvette (1 cm) containing potassium phosphate buffer *p*H 5.0 (50 μmole), the following was added: $MgCl_2$ (5 μmole); phosphoenolpyruvate (2 μmole); TPP (2 μmole); NADH (100 μg); pyruvate kinase (Boehringer, 20 μg); lactate dehydrogenase (Boehringer, 50 μg); rat brain mitochondrial suspension (200 μg of protein); fluid extract to be examined; and water to a final volume of 0.95 ml. The reaction was started by the addition of 0.05 ml of ATP (0.01*M*). The TPP was omitted from a control cuvette. The disappearance of NADH was followed at 340 nm for 10 to 15 minutes.

Addition	Change in adsorbancy $min^{-1} \times 10^3$
None	14.7 (25)‡
Normal blood*	14.5 (9)
SNE blood*	0.7 (9)
Father's blood*	8.6 (2)
Mother's blood*	14.5 (2)
Normal urine†	12.0 (12)
SNE urine†	0.5 (12)

* Protein was removed, equivalent to 0.1 ml of whole blood. † Salt was removed (see text), equivalent to 0.1 ml. ‡ Figures in parentheses indicate number of assays.

Table 2. Percentage distribution of thiamine compounds in tissues of normal patients and of patient with subacute necrotizing encephalomyelopathy (SNE). Protein was removed from tissue with perchloric acid. After removal of perchlorate with $KHCO_3$, a portion of the supernatant was placed on Munktell paper (No. S-311) and subjected to electrophoretic separation and fluorometric analysis (*5*). A, Patient died of congestive heart failure; B, patient died of influenza; C, patient died of cirrhosis of liver; D, patient died of renal and cardiac failure; and E, patient died of cancer. Abbreviations are: TTP, thiamine triphosphate; TPP, thiamine pyrophosphate; TMP, thiamine monophosphate; and T, thiamine.

Sample	TTP	TPP	TMP	T
	Cerebellum			
SNE	0.1*	78.0	8.0	14.0
	Frontal lobe			
SNE	0.1*	77.7	14.5	7.8
Normal (A)	12.4	72.3	4.3	11.0
Normal (B)	6.9	76.6	11.2	5.3
Normal (E)	9.9	58.0	14.8	17.3
	Liver			
SNE	9.7	76.2	5.1	9.0
Normal (B)	6.7	77.3	8.0	8.0
Normal (C)	4.9	74.4	8.5	12.2
Normal (D)	6.4	75.2	10.6	7.8
Normal (E)	6.5	63.6	14.0	15.9
	Kidney			
SNE	6.6	64.6	24.1	4.7
Normal (B)	5.1	69.4	9.2	16.3
Normal (C)	5.0	71.6	4.5	18.2
Normal (E)	8.6	47.3	19.4	24.7

* Below the limit of significance.

may be further support for a causal relation, as the father has had an unusual neurologic illness for nearly 30 years, originally diagnosed as "myositis." The blood of the patient's mother had no effect when tested against the phosphotransferase.

Of the total thiamine in the body, about 80 percent is in the form of TPP, about 10 percent is TTP, and the remainder is thiamine monophosphate (TMP) and thiamine (*6*). The coenzyme role of TPP is well documented, but virtually nothing is known about the function of TTP except for a report that it may be involved in the binding of TPP to an apoenzyme (*7*). However, in this study the activity of the three TPP-dependent enzymes in nervous tissue (pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and transketolase) was the same in the brain of the patient as the normal brain sample (*8*). A considerable amount of evidence has been accumulating to support the thesis that thiamine in some still unknown form has a specific role in ion movements in nervous tissue that is independent of its role as a coenzyme (*9*). It is thus conceivable that the neurophysiologically active form of the vitamin is TTP and that in SNE a factor is elaborated that inhibits the synthesis of TTP, the lack of which is ultimately reflected in the peculiar neurologic symptomatology that characterizes this condition.

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Traube-Hering Waves in the Pulmonary Circulation of the Dog

Abstract. *Although the pulmonary blood vessels receive an ample supply of nerves, it has been difficult to prove that these nerves operate in the regulation of the pulmonary circulation. In the present study, using the isolated lobe of the lung perfused in situ, waves in pulmonary arterial pressure were induced and were shown, as in the case of Traube-Hering waves in the systemic circulation, to be nervous in origin.*

The systemic arterial blood pressure is held remarkably constant by an elaborate system of control mechanisms. However, under certain experimental conditions, phasic and continuing fluctuations in systemic blood pressure occur either spontaneously or can be induced (*1*). One type of regular oscillations, synchronous with respiratory activity, was first described by Traube in 1865 (*2*) and subsequently by Hering (*3*). It has since been shown that these waves are nervous in origin and represent the phasic influence of respiratory activity on arterial and arteriolar tone produced by way of the vasomotor nerves (*4*).

The role of nerves in the control of the pulmonary circulation has been disputed for almost 100 years (*5*). If Traube-Hering waves did occur in the pulmonary circulation, they would constitute strong evidence in favor of a significant nervous control of the pulmonary blood vessels. However, waves in pulmonary arterial blood pressure have rarely been observed (*6*), and the opportunity for studying their genesis has been confined to the intact animal in which oscillations in systemic blood pressure coexisted (*7*). In a recent study in our laboratory (*8*), both systemic and pulmonary vasomotor waves were produced. However, it was not possible to prove that the vasomotor nerves to the pulmonary vessels were responsible for the genesis of these waves.

We have recently developed an isolated-lung preparation which proved to be ideal to settle the problem. This preparation makes it possible to perfuse, *in situ*, one lobe of a dog lung, by using levels and patterns of blood pressure and flow which approximate those which occur under natural conditions (Fig. 1). For the present experiments, a pulsatile flow pump was used to perfuse the left lower lobe with autologous blood. A tracheal divider separated the airway to the left lower lobe from the other airways; both sides were ventilated separately by constant-volume pumps. Airway pressure was recorded throughout the entire experiment and remained unchanged. The pulmonary artery and vein of the left lower lobe were cannulated with rigid L-shaped polyethylene tubing, and needles with three side holes (distal end obliterated) were passed through the walls of the cannula and connected to a strain gauge (Statham P23Db) to monitor blood pressure. Instantaneous flow was measured with a gated sine wave electromagnetic flow meter (Biotronex) by using cannulating transducers. The pulmonary vein cannula was connected to a water-jacketed blood reservoir which was kept at 37° to 38°C by a thermostated water bath. The level of the reservoir was monitored as hydrostatic pressure by a tap near the bottom which was connected to a Statham strain gauge (P23A) and calibrated for volume. In this preparation, stimulation of the sympathetic nerves to the lungs by way of the stellate ganglion elicits a characteristic pressor response, that is, an increase in systolic pressure but not in diastolic pressure (*9*).

With this preparation, synchronous fluctuations of systemic and pulmonary arterial blood pressure were induced during apneic oxygenation (by stopping, after preoxygenation, both mechanical

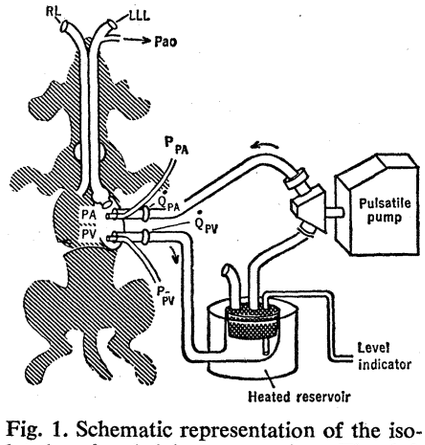

Fig. 1. Schematic representation of the isolated-perfused lobe preparation. The left lower lobe of a dog lung is perfused by a pulsatile pump, heated reservoir system, using autologous blood. A tracheal divider permits separate ventilation of the isolated lobe. *RL*, right lung; *LLL*, left lower lobe; *PA* and *PV*, cannulas in the left lower lobar artery and vein, respectively; P_{PA} and P_{PV}, pulmonary arterial and venous pressure taps; $\dot{Q}_{PA}$ and $\dot{Q}_{PV}$, electromagnetic flowmeters in series with the arterial and venous cannulas; P_{ao}, lateral tap on tracheal divider for measuring airway opening pressure.