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Role of Thiamine Triphosphate in Subacute Necrotizing Encephalomyelopathy

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Subacute Necrotizing Encephalomyelopathy (SNE) is a fatal, genetic disease in children that, until recently, was only diagnosed postmortem. Since the brain lesions in SNE are very similar to those in Wernicke's Encephalopathy, it had been suggested that SNE represented aberrant thiamine metabolism. We have recently found that extracts of spinal fluid, blood, and urine of patients with SNE inhibit a phosphoryl transferase in brain which catalyzes the synthesis of thiamine triphosphate (TTP) from thiamine pyrophosphate (TPP). This finding was supported by the subsequent observation that the brain of SNE patients at autopsy was essentially devoid of TTP, in contrast to normal brains. This inhibitor in the urine of SNE patients appears to be a protein of molecular weight around 30,000. The presence of this inhibitor in urine has been used successfully to diagnose SNE. Further, we have found that when patients with SNE are treated daily with large doses of either thiamine or thiamine propyl disulfide (a derivative that crosses cell membranes more readily than the vitamin) most patients exhibit marked temporary improvement.

Subacute Necrotizing Encephalomyelopathy (SNE, Leigh's Disease) is a genetic, degenerative disease in childhood which often becomes symptomatic in the first year of life, generally progressing to a fatal termination within a year. Signs of SNE are variable and include feeding problems, weakness, ocular palsy, loss of vision, difficulty in hearing and swallowing, ataxia, and peripheral neuropathy. The constellation of symptomatology is so varied that antemortem diagnosis of this disease is difficult and it has usually been diagnosed only by autopsy. On postmortem examination, lesions in the brain suggest a striking similarity to those seen in Wernicke's Encephalopathy, the classical thiamine deficiency disease. The similarities in clinical and pathological features of both diseases have suggested to clinicians that SNE represents some disorder or abnormality in thiamine metabolism. However, administration of thiamine in moderate amounts to patients with SNE has not resulted in any improvement in their condition. In addition, the enzymes which require thiamine as a coenzyme (pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and transketolase) have all been shown to have normal activity in the brain of a patient who died from SNE (Pincus *et al.*, 1969).

Before going into our studies on the etiology of SNE and possible treatment, it might be worthwhile to review some of the basic aspects of thiamine which we have undertaken in our laboratory.

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Over the past several years we have been accumulating information which supports our hypothesis that thiamine has a specific role in nervous tissue that is independent of its role as a coenzyme. These studies may be summarized as follows.

1. The electrophysiological effects of pyrithiamine, an antimetabolite of the vitamin, on the isolated rabbit vagus nerve have been shown to be due to a displacement of thiamine from the nerve, rather than to enzyme inhibition (Armett and Cooper, 1965; Cooper, 1968).
2. Thiamine is localized in nerve membranes rather than axoplasm, as shown by fluorescence histochemistry (Tanaka and Cooper, 1968).
3. Both electrical stimulation and neuroactive drugs release thiamine from a variety of intact nerve preparations (Cooper and Pincus, 1967; Itokawa and Cooper, 1970a). Neuroactive drugs also release the vitamin from nerve membrane fractions (Itokawa and Cooper, 1970b).
4. As nerve membranes are purified from a brain homogenate, the percentage of the thiamine triphosphate (TTP) form of the vitamin increases (Itokawa and Cooper, 1970b).
5. Thiamine restores the action potential in an ultraviolet irradiated nerve (Eichenbaum and Cooper, 1971).

There is a suggestion from our work that this neurophysiologically active form of thiamine may be thiamine triphosphate (TTP) in contrast to the coenzyme form, thiamine pyrophosphate (TPP). During the course of these studies we were interested in isolating and characterizing enzymes involved in thiamine metabolism. We isolated and partially purified thiamine pyrophosphatase from the brain (Cooper, 1970) and also isolated two new enzymes, thiamine triphos-

phatase (Hashitani and Cooper, 1972) and thiamine pyrophosphate–adenosine triphosphate phosphoryl transferase (Itokawa and Cooper, 1968). This latter enzyme, first described in yeast (Yusa, 1962), catalyzes the following reaction:

$$TPP + ATP \longleftrightarrow TTP + ADP$$

It is this enzyme with which we are concerned in our subsequent discussion of SNE.

METHODS

The TPP–ATP phosphoryl transferase was prepared and assayed as previously described (Itokawa and Cooper, 1968). The electrophoretic separation and fluorometric determination of thiamine and its phosphate esters was carried out by the procedure of Itokawa and Cooper (1970c). Total thiamine was determined by the procedure of Fujiwara and Matsui (1953). Protein was measured according to Lowry *et al.* (1951).

RESULTS AND DISCUSSION

About 2 years ago a child was admitted to the Yale–New Haven Hospital with the diagnosis of SNE. Her sister had died of the disease a year previously, so we were certain that this child also had the same problem. We took samples of her urine, blood, and spinal fluid and tested them on a variety of systems with which we were concerned at the time. To our surprise we found that extracts of all her body fluids inhibited the TPP–ATP phosphoryl transferase (Table I). There was no inhibitory effect on thiamine pyrophosphatase or thiamine triphosphatase nor could any effect be noted on release of thiamine from perfused nerve preparations. Normal blood and urine had no inhibitory effect on this enzyme.

Subsequently the child died and samples of her brain, liver, and kidney were processed to determine the thiamine content of these tissues and the form of the thiamine phosphate esters (Cooper *et al.*, 1969). It would have been predicted from our assay activity that the child would have very minimal amounts of thiamine triphosphate, since a factor in the child's blood, urine, and CSF inhibited the enzyme which synthesizes the triphosphate. Therefore it was not too surprising to find (Table II) that thiamine triphosphate was undetectable in the brain of the child compared to control brain tissue. The TPP–ATP phosphoryl transferase is also found in the liver and kidney and the usual amount of thiamine triphosphate was present in these tissues in the patient who died of SNE. A preliminary experiment in our laboratory indicated that the liver enzyme which makes thiamine triphosphate is not inhibited by this factor found in patients with SNE. This finding would explain the usual concentration of thiamine triphosphate in extraneural tissues. We have made a beginning attempt to characterize this inhibitory material. At the present time all we can say about it is that it is a protein, perhaps a glycoprotein, with a molecular weight in the neighborhood of 30,000. Since our original finding we have been

Table I. The Inhibition by Fluid Extracts from Patient with Subacute Necrotizing Encephalomyelopathy (SNE) of the TPP–ATP Phosphoryl Transferase of Rat Brain

Addition	o.d./min × 10^3
None	14.7 (25)[c]
Normal blood[a]	14.5 (9)
SNE blood[a]	0.7 (9)
Father's blood[a]	8.6 (2)
Mother's blood[a]	14.5 (2)
Normal urine[b]	12.0 (12)
SNE urine[b]	0.5 (12)

[a] Deproteinized, equivalent to 0.1 ml of whole blood. [b] Desalted, equivalent to 0.1 ml of original urine. [c] Figures in parentheses indicate number of assays. These data, as well as deproteinization, desalting, and assay procedures, have been previously reported (Cooper *et al.*, 1969; Pincus *et al.*, 1969). Reprinted with permission from *Science* **164**, 74 (1969).

Table II. Percentage Distribution of Thiamine Compounds in Tissues of Normal Patients and of Patient with Subacute Necrotizing Encephalomyelopathy (SNE)

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

[a] Below the limit of sensitivity. 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. E—Patient died of cancer. The data and methods of procedure have been previously reported (Cooper *et al.*, 1969; Pincus *et al.*, 1969). Reprinted with permission from *Science* **164**, 74 (1969).

Table III. Distribution of Thiamine and Its Phosphate Esters in Brain

Cause of death	Age of child	Brain sample	Total thiamine, μg/g	% of Total Thiamine			
				TTP	TPP	TMP	T
SNE[a]	4 months	Frontal lobe	7.40	0[b]	42.6	29.8	27.6
		Cerebellum	9.60	0	43.5	22.1	34.4
Malnutrition and battered child syndrome	4 years	Cortex	0.06	6.0	51.2	30.8	12.0
		Cerebellum	0.08	3.2	61.1	14.4	20.8
Mongolism congenital heart disease	(<1 mo) newborn	Cortex	0.24	8.7	61.7	15.7	13.9
		Cerebellum	0.52	8.6	59.0	22.8	9.5
Congenital heart disease	(1 mo) newborn	Cortex	0.60	7.2	53.0	30.9	9.0
		Cerebellum	0.87	10.0	42.0	25.0	23.0
Ventricular hemorrhage	(<1 mo) premature	Cortex	0.44	6.7	48.1	37.8	7.6

[a] This patient was on thiamine therapy which accounts for the high level of the vitamin. A patient who was untreated and died of SNE had a concentration of total thiamine in her frontal lobe of 0.39 μg/g and in the cerebellum of 0.40 μg/g. [b] Below the limits for accurate determination. This data has been published previously (Cooper *et al.*, 1970). Reprinted with permission from *New Engl. J. Med.* **283**, 793 (1970).

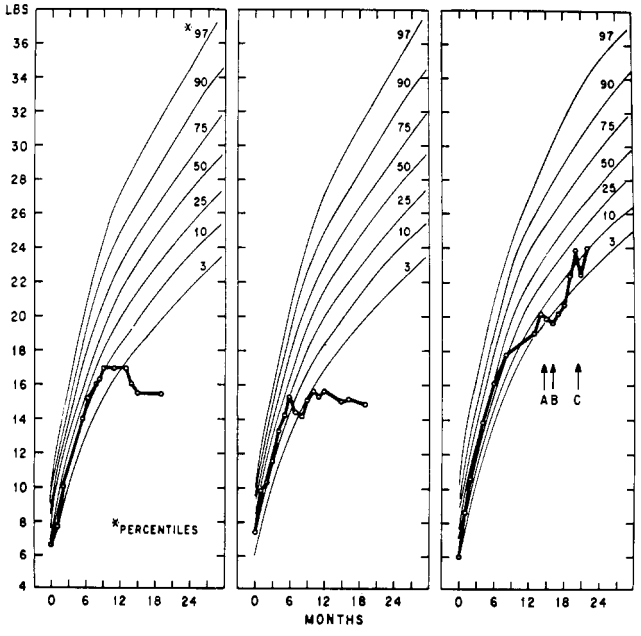

Figure 1. Weight charts of the three siblings with Subacute Necrotizing Encephalomyelopathy. The left-hand chart represents the first sibling; the middle chart is that of the second. The right-hand chart is that of the case reported here. Arrow "A" indicates the initiation of thiamine therapy, "B" indicates the time that daily doses of 1.5 g orally and 50 mg parenterally were achieved. Arrow "C" indicates the time at which thiamine propyl disulfide was started during his first hospitalization for respiratory failure. The neurological status of all three patients paralleled weight changes (from Pincus *et al.*, 1971). (Reprinted with permission from *Arch. Neurol.* 24, 511 (1971)

able to assay the brain of three other patients who died of SNE and in all cases low levels or a complete absence of TTP was noted in the brain. There is a suggestive correlation between the areas of severe lesions with absent or very low levels of TTP and *vice versa* (Pincus *et al.*, 1971). The proportion of total thiamine in the triphosphate form in the brains of young children or infants who died of a variety of diseases is identical to that of adults (Table III) (Cooper *et al.*, 1970). It is particularly interesting that one of the control values was from a child who was severely malnourished and although the total thiamine level of the child's brain was quite low, thiamine triphosphate was still present in fairly normal proportion.

Nonidentical twins were born to the parents of the first case we investigated. These children were perfectly normal at birth. When the twins were approximately 1 year old, one of them started showing slight ataxia and mild nystagmus. At this time we assayed the urine of both children and found that only the child who had exhibited ataxia had the inhibitory material in his urine. We thought that perhaps giving this child high doses of thiamine might overcome the inhibitory material and allow the synthesis of TTP to proceed in the brain. Accordingly the child was started on a high dose of the vitamin, which was gradually increased to 1500 mg per day. For the first 3 weeks the child seemed to maintain himself without going downhill in the usual course. After 3 weeks his appetite increased, he started to gain weight, and he became much more active. His ataxia disappeared and nystagmus diminished. The growth curves of this child and his two siblings who died of SNE are shown in Figure 1. A curious finding was that once the child was on thiamine therapy we could no longer detect the inhibitor in his urine. This observation has been substantiated in four other cases

Table IV. An Appraisal of the Specificity of the TPP–ATP Phosphoryl Transferase Assay for SNE

Patient	Age	Sex	Disease	Inhibitor assay
C. F.	12 mo	F	SNE	+
B. F.	12 mo	M	SNE	+
M. M.	2 yr	M	SNE	+
B. K.	7 yr	M	SNE	+
M. M.	11 yr	M	SNE	+
T. R.	4 mo	F	SNE	+
K. V.	1 yr	F	SNE	+
V. S.	4 yr	F	SNE	+
K. D.	12 yr	M	SNE	+
R. L.	6 yr	M	SNE	+
J. V.	7 yr	M	Probable SNE	+
S. L.	6 mo	M	Probable SNE	+
J. W.	13 mo	F	Probable SNE	+
L. V.	10 yr	F	Ataxia–myoclonus	+
C. H.	4 yr	F	Undine's Curse	+
E. D.	51 yr	F	Undine's Curse	+
S. H.	1.5 yr	F	Degenerative disease (?) etiology	−
W. M.	3 yr	M	Carbamyl phosphate transferase deficiency	−
G. M.	55 yr	M	Wernicke's Encephalopathy	−
E. W.	5 days	F	Werdnig–Hoffmann	−
J. T.	3 mo	M	Retardation and seizures	−
T. C.	7 yr	F	Seizures	−
K. M.	3 yr	M	Hemiballismus	−
S. B.	9 mo	F	Intermittent eye movement disorder	−
G. T.	64 yr	M	Progressive Supranuclear Ophthalmoplegia	−
J. G.	7 weeks	F	Failure to thrive (?) etiology	−
R. O.	12 yr	M	Ophthalmoplegia, heart block and retinitis pigmentosa	−
G. C.	25 yr	F	Multiple sclerosis	−
S. N.[a]	7 mo	F	Normal	+
T. F.[a]	12 mo	F	Normal	−
M. R.[a]	3 yr	M	Normal	+

Cases designated as "SNE" are based on either brain histopathology or a sibling with autopsy-verified SNE. Those designated as "Probable SNE" were diagnosed using clinical criteria only. Positive sign in inhibitor assay means >40% inhibition in the standardized assay.

[a] Normal sibling of patient with SNE.

who have been given high doses of thiamine therapy. That is, once they are treated with thiamine, no trace of inhibitor can be found.

When this child was about 18 months of age he suddenly had a relapse and was admitted to the hospital. The next morning he became apneic and depended on respiratory assistance delivered through a nasotracheal tube. His parents refused permission for a tracheostomy. On the third day of respiratory assistance it became necessary to remove the nasotracheal tube. It was expected that the child would die later on in the day, though thiamine propyl disulfide (TPD) in large doses was administered by continuous infusion. This compound, in contrast to thiamine, is transported by a passive diffusion process so that much higher blood and tissue levels of the vitamin can be achieved: TPD is converted to thiamine in the body (Suzuoki and Suzuoki, 1953). The child became much more alert and active, his respiratory rate increased to 12 per minute, although he still demonstrated marked tremor and nystagmus. By the next day the child was able to sit up in bed, hold a bottle, and recognize his parents. Intravenous therapy was continued for 2 weeks, at

which time the child was changed to oral thiamine propyl disulfide and discharged from the hospital. At the time of discharge, the child was essentially normal. The child remained well through the next 2 to 3 months, but improvement was not maintained beyond this without intravenous therapy. It became progressively more difficult to maintain a satisfactory spinal fluid concentration of thiamine. Discouraged by his failure to maintain improvement, his parents sought his admission to a small private hospital where all therapy was discontinued and he expired within 12 hr after admission (Pincus *et al.*, 1971).

Because spontaneous remissions have been reported in this disease, it is impossible to state absolutely that treatment with thiamine propyl disulfide and previous treatment with thiamine sustained this child's life. However, in comparison with his siblings, therapy did appear to have a beneficial effect. To our knowledge no one has reported a case of SNE in which a prolonged spontaneous remission followed a 3-day period of apnea. Apnea, in fact, is the usual mode of death.

Since this experience we have treated eight other children with thiamine or TPD. In six, remissions lasting from 3 months to 2 years were associated with therapy. In all four cases in which treatment was temporarily discontinued there occurred an exacerbation which appeared to respond to the reinstitution of the vitamin. Despite this, four of the six who enjoyed remissions ultimately died. The reasons for this are unknown but may relate to the induction of a cellular adaptation mechanism which prevents the maintenance of satisfactory concentrations of thiamine in spinal fluid over a period of several months. This has now been documented in one case in addition to that presented above.

Since we reported a diagnostic test for SNE we have had the opportunity to test many urine specimens received from patients with unusual neurological diseases from different medical centers. The results of these tests are shown in Table IV. There are several points of interest in this table. Since Leigh first described this disease in 1951, there have been 85 other cases of SNE reported. However, in the past year and a half we have detected ten cases of proven SNE (confirmed by autopsy or family history) and many other probable cases so that the disease, although rare, is not as uncommon as originally believed. Also, this inhibitor assay appears to be quite specific in that a wide variety of neurological diseases have been tested and shown to be negative with respect to the inhibitor assay. On the other hand, we have recently found urine inhibition in unusual neurological diseases of unknown etiology. These have included two cases of progressive ataxia associated with myoclonus and two patients with Undine's Curse (alveolar hypoventilation). In one of these, the patient's mother also had the inhibitor in her urine. This is particularly interesting to us since it has been reported that in this disease the lesions in the brain closely resemble those of SNE (Seriff, 1965). At the present time we are continuing this screening in order to appraise further the specificity of this inhibitor assay. It is conceivable that there are other variants of SNE which have yet to be classified where this inhibitory material is produced by affected individuals.

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