

# Simultaneous Separation and Estimation of Pyriethiamin and Thiamin Phosphate Esters in Tissues of Pyriethiamin Treated Rats

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## ABSTRACT

Pyriethiamin, a thiamin antagonist was separated from its phosphate esters for the first time, in the brain and liver of severely thiamin deficient rats (pyriethiamin-treated). Thiamin and its phosphate esters were also measured for comparison. It was concluded that pyriethiamin is phosphorylated in the same manner as thiamin, probably by the same enzymes.

**Key Words:** Rats; Pyriethiamin; Thiamin deficiency

## INTRODUCTION

The administration of thiamin antagonist, pyriethiamin, has been used extensively by many authors to produce acute thiamin deficiency in experimental animals. Pyriethiamin acts by inhibiting thiamin pyrophosphokinase, which catalyze the conversion of thiamin to its coenzyme form, thiamin pyrophosphate "TPP" (Johnson & Gubler, 1965; Steyn-Parve, 1967), and by itself phosphorylating to pyriethiamin pyrophosphate (Rindi & Perri, 1961) and thus suggested to competitively inhibit TPP-dependent enzymes (Steyn-Parve, 1967). Pyriethiamin is the preferred antagonist since it, unlike oxythiamin "another thiamin antagonist", crosses the blood brain barrier and accumulates in the brain causing clear neurological signs (Rindi & Perri, 1961; Seltzer & McDougal, 1974). Although pyriethiamin is suggested to be phosphorylated as mentioned earlier, no study in the literature could be traced on the distribution of pyriethiamin phosphate esters in pyriethiamin-treated experimental animals.

## MATERIALS AND METHODS

Thiamin deficiency was induced in 10 male albino rats as mentioned earlier (El Nageh, 1995). The rats were killed by decapitation, when signs of convulsions and opisthotonus were evident. Their brain and liver were removed at room temperature, blotted on a filter paper to remove excess blood, weighed and frozen in liquid nitrogen within 75 seconds and subsequently stored at -20°C. A weighed sample of brain or liver (0.6-0.9 g of brain or 0.5-0.7 g of liver) was homogenized in 15 ml of ice-cold 7.5% trichloroacetic acid and centrifuged for 45 minutes at 3000 rpm at 4°C. The supernatant was treated for fluorimetric measurement of thiamin (Gaitonde & Evans, 1983).

In a preliminary experiment, a portion (0.9 g) of the liver of a pyriethiamin treated rat that was injected subcutaneously with 350 n mol of thiamin containing 5 µCi of [thiazole-2-<sup>14</sup>C]

thiamin 20 minutes prior to death, was dissected and treated as above, and prepared for application on the columns for separation of thiamin and pyriethiamin phosphate esters. The eluates were collected in 5 ml fractions.

Thiamin and pyriethiamin was converted into fluorescent thiochrome, which was extracted with isobutanol before measurement of its fluorescence by an auto-analyzer. For thiamin, fluorescence was produced by excitation at 372 nm and emission was determined for thiamin at 420 nm. For pyriethiamin wave lengths of 420 nm for excitation and 470 nm for emission were used. The above wavelengths were arrived at after a series of experiments in which the excitation wavelength was fixed with varying the emission wavelength and vice versa. At the above wavelengths, there was no interference from pyriethiamin when thiamin was assayed and negligible interference from thiamin (approximately 1.5%) was observed when pyriethiamin was assayed using standards.

## RESULTS AND DISCUSSION

The elution profile shown in Fig. 1 indicated that the method used in this study provided a good separation of thiamin and its phosphate esters. It was assumed (Fig. 1) that pyriethiamin and its phosphate esters behaved on the column in the same way as their corresponding thiamin compounds. Thus each eluted fraction was examined for both thiamin and pyriethiamin compounds. An advantage of the assay conditions used in this study was the absence of any interference from pyriethiamin when assaying thiamin, and only negligible interference from thiamin (1.5%) when pyriethiamin was assayed. This interference from thiamin is insignificant considering that the concentrations of thiamin in the tissues of pyriethiamin treated animals was about one tenth that of pyriethiamin (Table I & II). A high recovery of thiamin compounds (96-100%) and of pyriethiamin compounds (96-98%) obtained by this method provided accurate assessment of the distribution of thiamin, pyriethiamin and their phosphate

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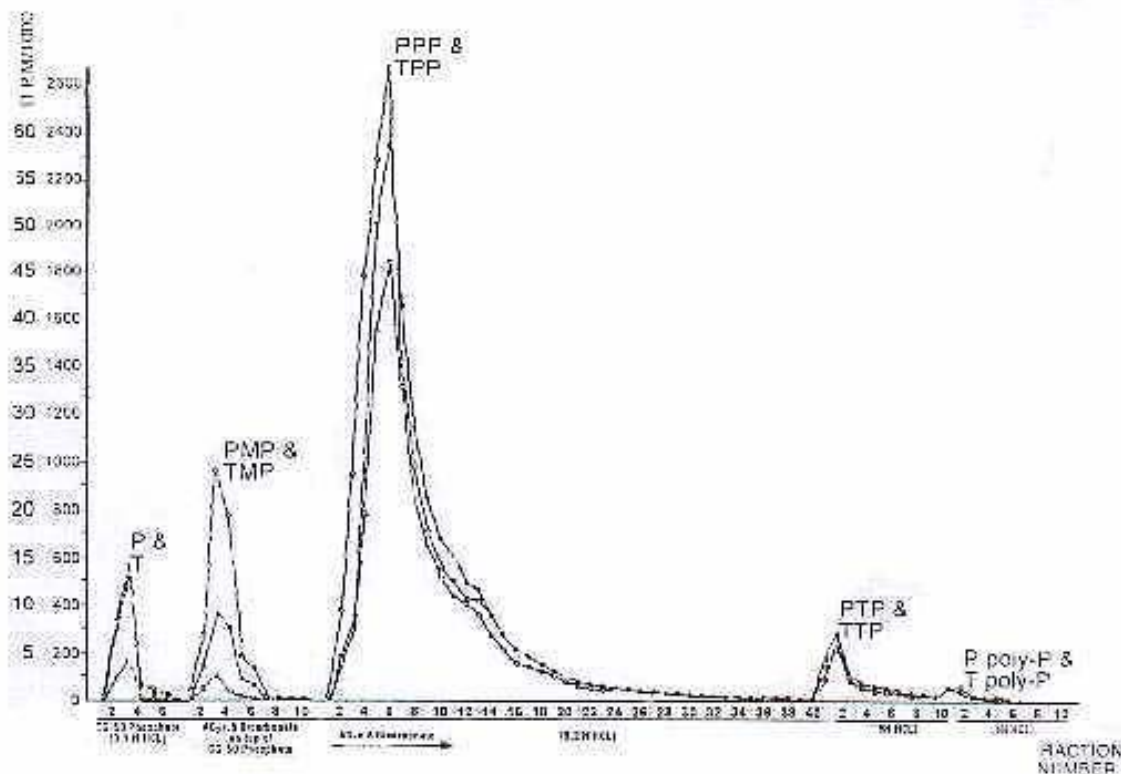


Fig. 1. Elution pattern of thiamin (O), pyriethiamin (□) and their phosphate esters and radioactivity content “DPM” of C-thiamin (●)  
 Abbreviations used: HAC= Acetic acid, HCL= Hydrochloric acid, DMP= Disintegrations per minute, PT= Free unphosphorylated pyriethiamin, PTMP= Pyriethiamin monophosphate, PTPP= Pyriethiamin pyrophosphate, PTTP= Pyriethiamin triphosphate, PT Poly-p= Pyriethiamin poly phosphate esters, T= Free unphosphorylated thiamin, TMP= Thiamin monophosphate, TPP= Thiamin pyrophosphate, T poly-P= Thiamin poly-phosphate esters

esters in rat tissues.

In earlier studies (Itokawa & Cooper, 1970; Patrini & Rindi, 1980), the recovery of thiamin was only 68-87% and

**Table I. Pyriethiamin and Pyriethiamin phosphate esters content in the brain and liver of thiamin-deficient (pyriethiamin-treated) rats (n=10)**

Chemicals	Pyriethiamin content (Mean ± SEM n mol/g tissue)	
	Brain	Liver
Total Pyriethiamin	8.22±0.52	28.61±2.00
PT	0.33±0.02	0.89±0.33
PT MP	1.28±0.020	3.39±0.79
PT PP	6.12±0.40	23.73±2.39
PT TP	0.17±0.06	0.25±0.02
PT poly-P	0.03±0.01	0.03±0.02

T= Free unphosphorylated pyriethiamin; PTMP= Pyriethiamin monophosphate; PTPP= Pyriethiamin pyrophosphate; PTTP= Pyriethiamin triphosphate; PT Poly-p= Pyriethiamin poly phosphate esters

on the recovery of pyriethiamin were traced out. Therefore, this is the first report on the separation of pyriethiamin and its phosphate esters. The pattern of distribution of free thiamin and its phosphate esters in the brain and liver reported in this study

(Table II) was in broad agreement with those obtained

**Table II. Thiamin and thiamin phosphate esters content in the brain and liver of thiamin-deficient (pyriethiamin-treated) rats (n = 10)**

Chemicals	Pyriethiamin content (Mean ± SEM n mol/g. tissue)	
	Brain	Liver
Total Thiamin	1.53±0.15	7.38±0.49
Free thiamin (T)	0.04±0.00	0.04±0.004
TMP	0.07±0.01	0.19±0.06
TPP	1.28±0.12	6.67±0.47
TTP	0.10±0.00	0.36±0.10
T poly-P	0.04±0.00	0.05±0.004

T= Free unphosphorylated thiamin; TMP= Thiamin monophosphate; TPP= Thiamin pyrophosphate; TTP= Thiamin triphosphate; T poly-P= Thiamin poly-phosphate esters

by other (Rindi & Giuseppe, 1961; Ishii *et al.*, 1979). The amount of pyriethiamin was higher in the liver than in the brain (Table I). This may be due to the daily administration of pyriethiamin, reflecting in an accumulation of pyriethiamin in the liver. In experiments after the administration of a single dose of pyriethiamin, the amount of pyriethiamin was found to increase with brain and decrease in other tissues, until it reached the

highest concentration in the brain after about 12 days (Rindi & Perri, 1961).

The results of this study are consistent with the view that pyrithiamin, like thiamin, participates in phosphorylation and dephosphorylation reactions in animal tissues (Table I). These reactions were probably catalyzed by the same enzymes. The proportion of pyrithiamin and its phosphate esters resembled with that of thiamin and its phosphate esters. The fact that pyrithiamin pyrophosphate constituted the highest proportion of pyrithiamin compounds, give support to the suggestion that pyrithiamin act by competing with TPP for enzymes binding site (Steyn-Parve, 1967).

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## REFERENCES

- El Nageh, K.M., 1995. Studies on the effect of thiamin deficiency on thiamin content and turnover in the rat brain. I. The effect of thiamin deficiency on distribution of thiamin and its phosphate esters in rat brain. *Tanta Med. J.*, 23: 123–33.
- Gaitonde, M.K. and G.M. Evans, 1983. Metabolism of thiamin *in vivo*. *Biochem. Soc. Transaction. 604<sup>th</sup> Meeting, Cambridge*, pp. 695–6.
- Ishii, K., K. Sarai, H. Sanemori and T. Kawaski, 1979. Concentration of thiamine and its phosphate esters in rat tissues determined by high performance liquid chromatography. *J. Nutr. Sci. Vitaminol.*, 25: 517–23.
- Itokawa, Y. and J.R. Cooper, 1970. Ion movements and thiamine. II. The release of the vitamin from membrane fragments. *Biochem. Biophys. Acta.*, 196: 274–84.
- Johnson, L.R. and C.J. Gubler, 1965. Studies with thiamine pyrophosphokinase from rat brain. *Federation Proc.*, 24: 481.
- Patrini, C. and G. Rindi, 1980. An improved method for the electrophoretic separation and fluorimetric determination of thiamin and its phosphates in animals tissues. *Int. J. Vit. Nutr. Res.*, 50: 10–18.
- Rindi, G. and L. De Giuseppe, 1961. A new chromatographic method for the determination of thiamin and its mono-, di- and tri-phosphates in animal tissues. *Biochem. J.*, 78: 602–6.
- Rindi, G. and V. Perri, 1961. Uptake of pyrithiamin by tissues of rat. *Biochem. J.*, 80: 214–6.
- Seltzer, J.L. and D.B. McDougal, Jr., 1974. Temporal changes of regional carboxylase levels in thiamine-depleted mouse brain. *American J. Physiol.*, 227: 714–8.
- Steyn-Parve, E.P., 1967. The mode of action of some thiamine analogues with antithiamine activity. In: *"Thiamine deficiency, Biochemical lesions and their clinical significances"* (Wolstenhome, G.E.W. and Oconor, M., Eds.). Ciba Foundation Study Group No. 28, pp. 26–53. J & A Churchill, London.

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