REFERENCES

[1] R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 52 (1952) 558, 565.
[2] J. D. SMITH AND R. MARKHAM, *Nature*, 170 (1952) 120.
J. D. SMITH AND R. MARKHAM, *Biochim. Biophys. Acta*, 8 (1952) 350.
[3] L. A. HEPPEL, P. R. WHITFIELD AND R. MARKHAM, *Biochem. J.*, 56 (1954) iii.
[4] CHONG-FU LI, W. G. OVEREND AND M. STACEY, *Nature*, 163 (1949) 538.
[5] See also, P. W. KENT, *Nature*, 166 (1950) 442.
[6] D. M. BROWN AND A. R. TODD, *J. Chem. Soc.*, (1952) 52.
[7] J. A. LUCY AND P. W. KENT, *Research*, 6 (1953) 49S.
[8] C. TAMM, H. S. SHAPIRO, R. LIPSCHITZ AND E. CHARGAFF, *J. Biol. Chem.*, 203 (1953) 673.
[9] C. TAMM, M. E. HODES AND E. CHARGAFF, *J. Biol. Chem.*, 195 (1952) 49.

Received April 28th, 1954

THE INFLUENCE OF FEEDING OXYTHIAMINE ON THE THIAMINE PYROPHOSPHATE CONTENT OF SOME TISSUES OF THE PIGEON

by

ELIZABETH P. STEYN-PARVÉ

Laboratory for Physiological Chemistry, The University, Utrecht (Netherlands)

The recent publication of a paper by DE CARO, RINDI AND GRANA[1] on the metabolic effects of (neo)pyrithiamine* and the thiamine contents in the tissues of the rat prompts us to communicate the results of some experiments, carried out in 1952, regarding the effect of feeding oxythiamine on the thiamine pyrophosphate (TPP) content of tissues of the pigeon. It was originally intended to publish these results as part of more extensive investigations on the mode of action of thiamine antagonists, but considering the interest this subject evokes in various quarters, it seems preferable not to delay this information.

Since the discovery in 1943 by WOOLLEY AND WHITE[3] that small doses of pyrithiamine, given to mice maintained on a diet containing adequate amounts of thiamine, produced symptoms closely resembling those of thiamine deficiency, evidence has accumulated pointing to the fact that structural analogues of thiamine, notably pyrithiamine and oxythiamine[4,5], act as antagonists to the vitamin *in vivo*. Judging from comparative studies, performed by CERECEDO and collaborators[6,7], it would seem that pyrithiamine has a more pronounced antivitamin effect than oxythiamine, as the former calls forth symptoms of polyneuritis in mice while the latter fails to do so, although typical symptoms have been observed in chicks fed oxythiamine[8].

WOOLLEY[9] has compared the TPP contents of the livers of 2 groups of mice, both fed a highly purified ration supplemented with thiamine and one group receiving a single dose of 0.5 mg pyrithiamine. Surprisingly, TPP was not reduced in the livers of the latter group as compared to the former, although the animals had developed severe symptoms of deficiency when they were sacrificed. Now in a deficiency provoked by deprivation of thiamine there is always a marked decrease of TPP in all tissues[10]. Although the precise mode of action of the antivitamin is as yet unknown, it is to be expected that it will somehow interfere with the replacement of TPP in the appropriate enzymes and if catabolism exceeds replacement, TPP will gradually disappear.

These considerations induced us to investigate this point. For practical reasons[11] pigeons were used as test animals and oxythiamine—much more easily obtainable than pyrithiamine—as antagonist**.

In the first experiment 2 groups of 6 pigeons each received—by forced feeding—2 g casein and 18 g sucrose daily, supplemented with salt and vitamin mixture[12] and 100 γ thiamine daily. In addition one group received 1 mg oxythiamine daily. After 15 days the animals were sacrificed and TPP was determined manometrically[13] in extracts of liver, heart muscle (left ventricle), breast muscle (pectoralis major) and brain (cerebrum). At this time the animals in the oxythiamine group did not yet show any pronounced symptoms of thiamine deficiency. Two animals in the control group receiving thiamine only had died from other causes. The average values and the ranges found

* In accordance with WOOLLEY's suggestion[2] the pure pyrithiamine will be called pyrithiamine and not neopyrithiamine.

** We wish to thank Dr J. LENS of N.V. Organon, Oss, for kindly supplying us with a sample of oxythiamine.

TABLE I

THIAMINE PYROPHOSPHATE CONTENT (in γ/g fresh tissue) OF SOME TISSUES OF PIGEONS RECEIVING THIAMINE AND OXYTHIAMINE, OR THIAMINE ONLY

Experiment 1: Group A. 4 pigeons, 100 γ thiamine daily.
Group B. 6 pigeons, 100 γ thiamine + 1 mg oxythiamine daily.
Experiment 2: Group A. 5 pigeons, 100 γ thiamine daily.
Group B. 7 pigeons, 100 γ thiamine + 2 mg oxythiamine daily.
Further details in text.

Exp. No.	*Group of pigeons*	*Cerebrum*		*Liver*		*Heart muscle*		*Breast muscle*	
		Av.	*Range*	*Av.*	*Range*	*Av.*	*Range*	*Av.*	*Range*
1	A	4.15	(3.80–4.55)	5.35	(3.90–6.30)	6.70	(4.70–7.70)	6.25	(5.45–7.05)
	B	3.75	(3.00–4.25)	4.90	(3.00–5.65)	3.40	(2.00–4.70)	4.60	(2.95–6.10)
	P value		0.215		0.352		0.014		0.038
2	A	4.35	(3.85–4.65)	5.10	(4.60–5.45)	5.85	(5.35–6.15)	7.60	(7.10–8.30)
	B	3.95	(3.40–4.50)	4.70	(4.05–5.55)	3.85	(3.10–5.30)	6.40	(5.55–7.55)
	P value		0.174		0.343		0.001		0.030

for the TPP contents of the afore-mentioned tissues in the 4 controls and the 6 pigeons receiving oxythiamine are assembled in Table I. As is borne out by the P values calculated according to WILCOXON there is a significant decrease of TPP in heart and breast muscle in the animals receiving oxythiamine. The slight decrease in liver and brain is not significant.

As is also shown in Table I, a second experiment, performed in the same way, fully confirmed the first results. This time 5 animals in the control group and 7 animals in the oxythiamine group were examined. The latter now received 2 mg oxythiamine daily and the feeding period lasted 18 days, but this made no essential difference.

It appears from these experiments that feeding oxythiamine does result in a decrease of TPP in some tissues. Of the tissues examined, heart muscle and breast muscle are most susceptible to the influence of oxythiamine. The liver shows no significant response to the feeding of the antivitamin, in agreement with WOOLLEY's observations, although it seems doubtful whether the effects of oxythiamine and pyrithiamine may be compared directly with one another. In this respect it is intriguing that DE CARO *et al.*[1] do find a decrease of total thiamine in the liver after feeding pyrithiamine to rats. However, their dose considerably exceeds that given by WOOLLEY to his mice, also proportionally.

It is not surprising that we observed no deficiency symptoms. In heart muscle and breast muscle TPP had only declined to the level attained after 4 days of thiamine deprivation, using the same basal diet[10], and symptoms usually do not occur then before the thirteenth day.

This work forms part of investigations on the metabolism and physiological function of thiamine carried out by H. G. K. WESTENBRINK and collaborators.

REFERENCES

1 L. DE CARO, G. RINDI AND E. GRANA, *Experientia*, 10 (1954) 140.
2 D. W. WOOLLEY, *A Study of Antimetabolites*, New York, 1952.
3 D. W. WOOLLEY AND A. G. C. WHITE, *J. Biol. Chem.*, 149 (1943) 285.
4 M. SOODAK AND L. R. CERECEDO, *J. Am. Chem. Soc.*, 66 (1944) 1988; *Federation Proc.*, 6 (1947) 293.
5 C. E. FROHMAN AND H. G. DAY, *J. Biol. Chem.*, 180 (1949) 93.
6 A. J. EUSEBI AND L. R. CERECEDO, *Science*, 110 (1949) 162.
7 L. R. CERECEDO, M. SOODAK AND A. J. EUSEBI, *J. Biol. Chem.*, 189 (1951) 293.
8 L. J. DANIEL AND L. C. NORRIS, *Proc. Soc. Exptl. Biol. Med.*, 72 (1949) 165.
9 D. W. WOOLLEY, *J. Biol. Chem.*, 191 (1951) 43.
10 C. H. MONFOORT, *Biochim. Biophys. Acta*, 9 (1952) 331.
11 M. GRUBER, *Nature*, 166 (1950) 78.
12 M. GRUBER, *Acta Physiol. Pharmacol. Neerl.*, 2 (1951–1952) 411.
13 H. G. K. WESTENBRINK AND E. P. STEYN-PARVÉ, *Int. Rev. Vitamin Res.*, 21 (1950) 461.

Received May 12th, 1954