The influence of thiamine on nitrogen metabolism

Neopyrithiamine, an antivitamin for thiamine in rats[1] and mice[2], has been found to produce a marked accumulation of glutamic acid, and simultaneously a considerable fall in the aspartic acid synthesized during the germination of green gram (*Phaseolus radiatus*) seeds[3]. These results suggested a conversion of glutamic acid to aspartic acid and asparagine during germination according to the sequence of reactions:

Glutamine → Glutamic acid → α-ketoglutaric acid → Succinaldehyde → Succinic acid → Fumaric acid → Malic acid → Oxalacetic acid → Aspartic acid → Asparagine.

Neopyrithiamine inhibits this conversion by interfering with the decarboxylation of α-ketoglutaric acid to succinaldehyde. In subsequent investigations with ^{14}C-labelled glutamic acid[4], it has been shown that radioactivity is incorporated into asparagine from glutamic acid, and that neopyrithiamine inhibits this incorporation. In the present study, the influence of neopyrithiamine on the levels of the amides, glutamine and asparagine, as well as free ammonia during germination, and also the abilities of thiamine and cocarboxylase to reverse the changes due to neopyrithiamine have been investigated.

The effects of neopyrithiamine on the levels of glutamine, asparagine and free ammonia in green gram seeds were studied by carrying out germinations with 5 g lots of seeds in 11 cm petri dishes with media containing (1) water only (Control), (2) 0.7 mg neopyrithiamine and (3) 1.0 mg neopyrithiamine, the total volume in all cases being adjusted to 24 ml with sterile water. At the end of 72 h germination in diffuse light, the seedlings were washed, ground with cold water, and centrifuged. The extract was removed, and the residue re-extracted twice with cold water. The combined extracts were then deproteinised by the addition of a few drops of glacial acetic acid just to start the coagulation of the protein (pH 5.0 to 5.5), and then by rapid heating to 90° C, when the precipitation was complete. The solution was cooled immediately, and then made up to a known volume. Glutamine, asparagine and free ammonia in this solution were estimated according to VICKERY, PUCHER, CLARK, CHIBNALL AND WESTALL[5]. A slight destruction of glutamine with a corresponding increase in free ammonia may take place during the few minutes of heat coagulation, which, however, is negligible, where only comparisons are made. Also, as will be seen later, the changes produced by neopyrithiamine on free ammonia are too large to allow any small error in this operation to vitiate the correct interpretation.

Germinations with total medium containing (1) water only, (2) 1.0 mg neopyrithiamine, (3) 1.0 mg neopyrithiamine + 0.5 mg thiamine and (4) 1.0 mg neopyrithiamine + 1.0 mg cocarboxylase, were conducted to study the counteracting abilities of thiamine and cocarboxylase. High concentrations of thiamine hydrochloride (2.5 mg per ml) and cocarboxylase (1.5 mg per ml) were found to be toxic by themselves, probably due to their acidic nature.

From Table I, it will be seen that neopyrithiamine produces a considerable lowering in asparagine. Also, there is a simultaneous increase in free ammonia. Neopyrithiamine probably does not inhibit the production of ammonia by the deamination of various amino acids, but interferes only with its normal utilisation. The accumulation of ammonia may be due to a lack of adequate production of oxalacetic acid to fix it as asparagine. If, as suggested earlier[3], neopyrithiamine inhibits the conversion of α-ketoglutaric acid to succinaldehyde during the formation of asparagine, a paucity of oxalacetic acid could be expected. This would account for the accumulation of ammonia, and the fall in asparagine. It is rather surprising to note that, while wide variations occur in the levels of asparagine and free ammonia, the glutamine level remains remarkably constant. Considering the marked accumulation of glutamic acid noted earlier[3], one would expect the glutamine level also to register a corresponding increase. But this is not so, though its immediate precursors, glutamic acid and ammonia, do show an increase. This can happen if, owing to impaired energy metabolism caused by neopyrithiamine interference with the Krebs cycle, there is a lack of production of adenosine triphosphate, which is known to be necessary for glutamine synthesis in seedlings[6].

TABLE I

THE EFFECT OF NEOPYRITHIAMINE ON THE AMIDES AND FREE AMMONIA IN GREEN GRAM SEEDS

(Values in mg per 5 g seeds)

	Control	*Neopyrithiamine*	
		0.7 mg	*1.0 mg*
Asparagine	70.2	10.07	11.41
Glutamine	29.85	30.75	30.28
Free ammonia	0.7577	3.111	2.735

The effects of neopyrithiamine on free ammonia and the amides are partially reversed by thiamine, while cocarboxylase is much more effective and brings about nearly complete reversal (Table II). On this basis, it is concluded that the changes observed with neopyrithiamine are *specifically* due to its "antivitamin" property in this species also. It has also been observed that while, at the dosage employed, cocarboxylase nearly completely reverses the changes in asparagine and free ammonia, it is only slightly effective in restoring the growth inhibited by neopyrithiamine. This shows that the changes observed in the nitrogeneous constituents are primarily due to a state of thiamine deficiency, and not due to growth inhibition. The striking changes produced by neopyrithiamine in the levels of glutamic acid, aspartic acid, asparagine and ammonia, substances which are all closely linked with the Krebs tricarboxylic acid cycle, seem to point significantly to the influence, direct or indirect, which thiamine exerts on nitrogen metabolism.

TABLE II

THE REVERSAL OF NEOPYRITHIAMINE-EFFECT BY COCARBOXYLASE AND THIAMINE

(Values in mg per 5 g seeds)

	Control	*Neopyrithiamine 1 mg*	*Neopyrithiamine 1 mg + cocarboxylase 1 mg*	*Neopyrithiamine 1 mg + thiamine 0.5 mg*
Free ammonia	0.67	2.41	0.6323	1.471
Glutamine	29.09	32.42	30.28	35.76
Asparagine	85.45	19.15	70.78	30.23

Indirect evidence for the absolute requirement of thiamine in glutamic acid metabolism has been obtained in animals also. While normally-fed rats tolerated well an intraperitoneal injection of sodium glutamate, neopyrithiamine-treated rats, showing no other symptoms of vitamin deficiency than weight loss, developed immediately after injection of glutamate all the symptoms of acute thiamine-deficiency—hunched back, staggered gait, severe paralysis of the limbs, violent convulsions, the animals rolling on their sides and flinging themselves in the air, ending finally in death, and all this occurring within an hour of the injection. The same rats had, three hours earlier, withstood well an injection of the same volume of sterile water, so that the effect is not due to the shock of the injection, but to the glutamate injected. This aggravation of thiamine deficiency symptoms by glutamic acid is interpreted as being due to a complete utilization, and hence exhaustion, of all the available thiamine for the metabolism of the glutamic acid injected.

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V. M. SIVARAMAKRISHNAN
P. S. SARMA

University Biochemistry Laboratory, Madras (India)

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The estimation of cysteine and cystine by potentiometric titration with mercuric chloride

Methods have so far been published for the estimation of thiols and disulphides by amperometric titration with copper (II)[1], silver[2,3,4] and mercury (II)[4,5,6] and by potentiometric titration with silver[7]. It has now been found that potentiometric titration with mercuric chloride offers sufficient advantages to justify the introduction of yet another method. In this short communication the results with cysteine and cystine are described.