

A NEW UNDERSTANDING OF THE ROLE OF VITAMIN B₁₂ IN METHYLATION
OF PROTEINS IN THE NERVOUS SYSTEM

D.H. SMALL* and P.R. CARNEGIE*

Vitamin B₁₂ deficiency is often accompanied by a neurological condition known as subacute combined degeneration (SCD). SCD is characterized by a spongiform vacuolation of myelin in the central and peripheral nervous systems. Abnormalities in fatty acid metabolism have been suggested as the cause of the lesions, but the experimental evidence is not convincing. Since nitrous oxide has recently been shown to induce SCD, this emphasizes the role of methylcobalamin in the maintenance of myelin (Reed *et al.* 1979). Methylcobalamin is required to maintain adequate levels of methionine in brain.

Cycloleucine has been shown to cause lesions in rats and mice which are identical to SCD (Ramsey and Fischer 1978) and to interfere with methyl group metabolism by inhibiting the formation of S-adenosylmethionine. S-adenosylmethionine is required for the methylation of arginine in myelin basic protein (Baldwin and Carnegie 1971).

Two intraperitoneal injections, each of 40 mg cycloleucine, into young chickens caused, over the first two weeks, neurological disturbances and a marked depression in growth rate, which could be counteracted by an increase in the methionine content in the diet. Brains from affected chickens showed lesions typical of SCD. Using a double isotope procedure, the effect of cycloleucine on the *in vivo* incorporation of methyl labelled methionine into myelin was studied. Chickens were injected intracranially with 20 μ Ci of ³H or ¹⁴C methyl-labelled methionine on day-8 after hatching, and were killed 6h later. Hydrolysis of myelin and separation of labelled amino acids showed that incorporation of methyl groups in to N^G, N¹G-dimethyl- and N^G-monomethylarginine (sDMA and MMA) was markedly inhibited (P<0.05) (Table 1).

TABLE 1. Effect of cycloleucine on methyl incorporation into methyl amino acids of myelin

	Methionine	sDMA	MMA
% Inhibition by cycloleucine	2.9	41.3	34.6
S.E.M. and No. of Determinations ()	1.6 (5)	6.9 (4)	7.2 (5)

Both sDMA and MMA were found to be derived solely from myelin basic protein. In an earlier experiment where total methylarginines were measured, a 32% decrease in methylation was observed.

Cycloleucine toxicity provides a good model for examining the role of methylcobalamin in maintaining the structural integrity of the myelin sheath and the role of protein methylation in membrane function.

BALDWIN, G.S. and CARNEGIE, P.R. (1971) *Science* 171 : 579.
 RAMSEY, R.B. and FISCHER, V.W. (1978) *J. Neurochem.* 30 : 447.
 REED, B., DINN, J., MCCANN, S., WILSON, P., O'SULLIVAN, H., WEIR, D.G. and SCOTT, J.M. (1979) In Vitamin B₁₂, p. 1061 (B. Zagalak and W. Friedrich, eds.). Berlin : Walter de Gruyter.

* School of Agriculture, La Trobe University, Bundoora, Victoria. 3083.