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The role of methionine and vitamin B_{12} in folate incorporation by rat liver

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The decreased folate uptake induced by exposure of rats to nitrous oxide can be partly reversed by methionine supplementation. This is consistent with the theory that N_2O inhibits methylcobalamin-dependent methionine synthetase (*EC* 2.1.1.13) in rat liver and previous observations showing decreased folate incorporation in rats made nutritionally vitamin B_{12} deficient.

The effects of vitamin B_{12} deficiency on folate metabolism are now reasonably well documented in experimental animals. Elevated excretion of formiminoglutamic acid (FIGlu) and formate has been demonstrated in rats fed on a diet low in both vitamin B_{12} and methionine (Silverman & Pitney, 1958). Accompanying this is a reduction in the amount of folate retained by the liver and an alteration in the relative concentrations of its different forms (Noronha & Silverman, 1962; Kutzbach *et al.* 1967).

The administration of methionine to vitamin B_{12} deficient animals appears to restore folate metabolism to normal or near normal even in the absence of vitamin B_{12}. It was originally shown to decrease FIGlu excretion by Silverman & Pitney (1958). Subsequently many workers have reported that methionine supplementation of vitamin B_{12}-deficient rats either by dietary means or by injection restores liver folate concentration and distribution to normal or near normal levels (Noronha & Silverman, 1962; Gawthorne & Stokstad, 1971). Most diets used for producing vitamin B_{12} deficiency also produce a partial methionine deficiency and the effect of adding methionine to a pure vitamin B_{12}-deficient diet has not been fully explored as yet.

Vitale & Hegsted (1969) showed that in the presence of adequate amounts of methionine no effect of vitamin B_{12} deficiency on folate uptake could be seen. However, they postulated that the limited amounts of vitamin B_{12} available to the body even during vitamin B_{12} deficiency were adequate for other purposes when the need for methyl group synthesis was removed. The need would seem to exist, therefore, for a method of investigating methionine supplementation in an animal given normal amounts of dietary methionine but functionally deficient with respect to vitamin B_{12}.

McGing *et al.* (1978) have recently shown the inhibition of vitamin B_{12}-dependent demethylation of 5-CH_3-H_4 pteroylglutamate by nitrous oxide in mouse liver in vivo in addition to decreased liver polyglutamate biosynthesis and folate incorporation. Similar observations were made in rats. Subsequent work (Deacon *et al.* 1978; Scott *et al.* 1979) has verified that N_2O represents an efficient method of producing functional vitamin B_{12} deficiency in experimental animals with none of the drawbacks of dietary manipulation.

The aim of this study was to extend the use of this experimental system to examine the effects of methionine supplementation on liver folate incorporation by rats with functional vitamin B_{12} deficiency but with normal nutritional status with respect to methionine.

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MATERIALS AND METHODS

Radiochemical and chemicals. The radiochemical pteroylglutamate (PteGlu), potassium salt, labelled in the 3',5',9 positions (48 Ci/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks. The L-methionine was obtained from BDH Chemicals Ltd, Poole, Dorset.

Induction of functional vitamin B_{12} deficiency using N_2O. Animals were placed in sealed chambers through which was flowing N_2O–oxygen (50:50, v/v) for 24 h at a flow-rate of 0·5 l/min (McGing *et al.* 1978).

Animals. Adult Albino Wistar rats (approximately 200 g) were divided into two groups: A, normal controls; B, vitamin B_{12}-inactivated. Each of these groups was further divided into three sub-groups which were given either 0, 40 or 120 mg L-methionine intraperitoneally. This was followed immediately by 20 μCi [^{3}H]PteGlu. Rats in group B were placed in N_2O—O_2(50:50, v/v) for 1 h before injection with [^{3}H]PteGlu and returned to that environment for a further 24 h before slaughter. The control rats were left in air for the same period. Food and water were supplied *ad lib.* to both groups. Animals were killed by cranial fracture and their livers removed immediately onto a chilled surface and weighed.

Measurement of liver folate incorporation. Folates were extracted by the method of Houlihan & Scott (1972). ^{3}H was measured in a Packard Tri-Carb Liquid-Scintillation Counter (Model 3385). Scintillation fluid of toluene-Triton-X-100 (2:1, v/v) containing 2·667 g PPO/l was used as a scintillant (10:1, v/v). Quenching was determined by the use of an internal standard. Total counts incorporated were determined and significance was calculated using the Student's *t* test.

RESULTS AND DISCUSSION

It has been postulated that in vitamin B_{12} deficiency inactivity of the methylcobalamin-dependent methionine synthetase (*EC* 2.1.1.13) results in folate being trapped as 5-CH_3-H_4PteGlu (Herbert & Zalusky, 1962; Noronha & Silverman, 1962). Subsequent studies have suggested that methionine controls entry of folate into this so-called methyl folate trap via S-adenosylmethionine (SAM)-mediated inhibition of 5,10-CH_2-H_4PteGlu reductase (*EC* 1.1.1.68) (Kutzbach & Stokstad, 1967).

In previous studies with dietary induction of vitamin B_{12} deficiency most workers have shown a 55–65% reduction in rat liver folate incorporation (Kutzbach *et al.* 1967; Gawthorne & Stokstad, 1971; Davidson *et al.* 1975), with one report of 75% reduction (Shane *et al.* 1977). Methionine supplementation of vitamin B_{12}-deficient animals, either by dietary means or by injection, reversed this reduction with folate incorporation often being increased to near-normal levels (Vitale & Hegsted, 1969; Williams & Spray, 1976; Chiao & Stokstad, 1977). Gawthorne & Stokstad (1971) reported no effect on [^{3}H]PteGlu uptake of 40 mg methionine injections to rats deficient in both vitamin B_{12} and methionine. They also reported a dramatic effect of 225 mg methionine (bringing the ^{3}H incorporation to 160% of the control value). In the latter instance no further increase was obtained with supplemental vitamin B_{12}. In addition there was no effect of 40 mg methionine injected into control rats but with 225 mg injection there was a difference ($P < 0.01$).

We investigated liver incorporation of [^{3}H]PteGlu by vitamin B_{12} inactivated and control rats with and without methionine supplementation (Table 1). In confirmation of previous findings in mice (McGing *et al.* 1978) later extended to rats (McGing, unpublished results), folate uptake was found to be markedly reduced in vitamin B_{12}-inactivated rats, the reduction being of the order of 80%. No significant effect was seen when injecting control rats with either 40 or 120 mg methionine, indicating that excess methionine at the level given did not in itself have an effect on liver folate incorporation. Similar injections when given to

Table 1. *Effect of methionine on rat liver incorporation (nCi/g) of injected [^3H]pteroylglutamic acid (PteGlu)*

(Mean values with their standard errors; no. of animals in parentheses)

Group ...	1	2	3
Methionine administered (mg)	0	40	120
Controls	88±9 (11)	102±12 (7)^{NS}	97±9 (8)^{NS}
Vitamin B_{12}-inactivated	19±2 (9)*	31±4 (9)*†	29±4 (15)*††

NS, values not statistically significantly different from group 1.
* Values were statistically significantly different from the corresponding control group ($P < 0.001$). Values were statistically significantly different from group 1: †$P < 0.02$, †† $P < 0.05$.

vitamin B_{12}-inactivated rats produced small but statistically significant increases in folate incorporation. No significant difference was shown either in the control or vitamin B_{12}-inactivated rats between injecting with 40 or 120 mg methionine.

We believe that the greater reduction of folate incorporation achieved in this study to be due to a more complete vitamin B_{12} deficiency situation being produced functionally with N_2O than was the case with dietary methods. Our results indicate that even in the presence of N_2O some folate is demethylated and stored within the liver. Although injected PteGlu is believed to be converted to 5-CH_3-H_4PteGlu for transport to the liver (Herbert *et al.* 1962) and would therefore be available to liver cells as 5-CH_3-H_4PteGlu, we cannot be certain that the liver would not be presented with any other form of folate.

The effect shown of methionine supplementation on vitamin B_{12}-inactivated rats is consistent with the introduction of an extra control of folate entry into the trap. However, the conclusive finding of the inability of methionine to restore liver folate incorporation to greater than one-third of normal indicates strongly that methionine cannot enable cellular folate to be converted to a pteroylpolyglutamate once trapped as 5-CH_3-H_4PteGlu. It is probable that if a total inhibition of vitamin B_{12} could be achieved, together with cellular uptake of 5-CH_3-H_4PteGlu alone, folate incorporation would be negligible and methionine supplementation would have no effect on liver PteGlu incorporation.

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