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Cobalamin inactivation induces formyltetrahydrofolate synthetase

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Loss of cobalamin function produces profound changes in the metabolism of formate. There is impaired synthesis of formyltetrahydropteroylglutamate synthetase (CHO-H_4PteGlu), accumulation of endogenous formate and impaired utilization of [^{14}C]formate. There are contradictory reports on the effect of cobalamin inactivation on CHO-H_4PteGlu synthetase. This study confirms a significant increase in synthetase activity following cobalamin inactivation.

Cobalamin; Formate metabolism; Formyltetrahydrofolate synthetase

1. INTRODUCTION

Oxidation of cob(I)alamin by nitrous oxide leads to rapid loss of methionine synthetase activity in all tissues in which it has been measured [1]. The biochemical effects of Cbl inactivation are reversed by either giving methionine, 5′-methylthioadenosine which is a product of methionine metabolism concerned with polyamine synthesis, or 5-CHO-H_4folate [2]. All 3 compounds are effective in so far as they supply 'active' formate [3] and hence it has been suggested that Cbl, by virtue of its role in the de novo synthesis of methionine, is concerned in the provision of 'active' formate [4].

Defects in formate metabolism that are present when Cbl is inactivated include increased levels of endogenous formate in blood, liver [5] and brain, increased urinary loss of formate [6], impaired utilization of [^{14}C]formate in all 1-carbon unit transfers [7], very low tissue levels of CHO-H_4folate [8] and induction of the enzyme responsible for the synthethis of CHO-H_4folate, formyltetrahydrofolate synthetase [9].

We were very surprised to see a report that failed to demonstrate any increase in CHO-H_4folate synthetase in livers of rats exposed to N_2O [10]. We have therefore repeated studies we carried out 10 years ago to reassess the effect of N_2O on this enzyme.

2. MATERIALS AND METHODS

2.1. *Animals*

Male, Sprague–Dawley rats weighing 80–100 g were used. The animals were housed in a perspex environmental chamber and exposed to a mixture of N_2O/O_2 (1:1). At the beginning of each experiment the gas was flushed through the chamber at a flow rate of 6 litres/min for 20–30 min. Thereafter, the gas concentration was maintained at a constant level via a diaphragm pump using a flow rate of 0.5 litres/min; CO_2, humidity and temperature were all controlled. Control animals were kept in air. The animals were killed by exsanguination following an overdose of sodium pentabarbitone. Livers were removed and prepared as previously described [9].

2.2. *Enzyme assays*

The activity of formyltetrahydrofolate synthetase (EC 6.3.4.3) was determined by the method of Rabinowitz and Pricer [11] and also using the modifications described by Appling and Rabinowitz [12]. Protein concentrations were estimated by the method of Lowry et al. [13].

2.3. *Statistical analysis*

This was carried out using one-way analysis of variance (ANOVA) to test the group means followed by Dunnett's method [14] for multiple comparison of means using the pooled SD calculated as part of ANOVA.

3. RESULTS

The results (Table I) show that exposure to N_2O was accompanied by a significant increase in CHO-H_4folate synthetase activity. The method of assay did not affect the pattern of the result.

4. DISCUSSION

Our previous report [9] involved a detailed and careful study with over 100 animals following the increase of CHO-H_4folate synthetase during Cbl inactivation and the return to normal activity with recovery of Cbl function. Ten years later we confirm these data. We cannot explain the failure of Barlowe and Appling [10] to duplicate our results. We have used locally bred Sprague–Dawley rats but rarely we have obtained odd results when rats from other sources were used. This in-

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Abbreviations: Cbl, cobalamin; CHO-H_4PteGlu, formyltetrahydropteroylglutamate synthetase; MTA, 5′-methylthioadenosine; N_2O, nitrous oxide

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Table I

Formyltetrahydrofolate synthetase activity in rat liver before and after exposure to N_2O (nmol 5,10 CHO-H_4folate/min per mg protein)

		Method A	Method B
Controls	(5)	0.50 ± 0.10	0.88 ± 0.22
1 day N_2O	(3)	0.98 ± 0.11**	1.30 ± 0.04*
2 day N_2O	(3)	1.23 ± 0.07**	1.62 ± 0.21**

Method A: Rabinowitz and Pricer [11]; Method B: Appling and Rabinowitz [12]. Numbers of animals in parentheses.
Values statistically significant from controls: *$P<0.05$; **$P<0.01$.

cludes a single animal that did not show a rise in synthetase activity on N_2O exposure. The concentration of N_2O and flow rate are of little importance provided that more than trace amounts of N_2O are used. N_2O rapidly penetrates cells and its effect on Cbl is rapid. However, recovery of, say, methionine synthetase activity on N_2O withdrawal takes 3 or more days as not only must Cbl be replaced but fresh apoenzyme is required as the protein is damaged by release of active oxygen radicals.

Should the difference in Barlowe and Appling [10] results be due to a resistant rat, the mechanism would be of great interest.

REFERENCES

[1] Chanarin, I., Deacon, R., Lumb, M., Muir, M. and Perry, J. (1985) Blood 66, 479–489.
[2] Perry, J., Chanarin, I., Deacon, R. and Lumb, M. (1983) J. Clin. Invest. 71, 1183–1190.
[3] Trackman, P.C. and Abeles, R.H. (1981) Biochem. Biophys. Res. Commun. 103, 1238–1244.
[4] Chanarin, I., Deacon, R., Lumb, M. and Perry, J. (1989) Blood Rev. 3, in press.
[5] Deacon, R., Perry, J., Lumb, M. and Chanarin, I. (1990) Br. J. Haematol., in press.
[6] Stokstad, E.L.R., Webb, R.E. and Shah, E. (1966) J. Nutr. 88, 225–232.
[7] Deacon, R. (1989) PhD Thesis, Council for National Academic Awards, UK.
[8] Lumb, M., Perry, J., Deacon, R., Chanarin, I. (1981) Am. J. Clin. Nutr. 34, 2412–2417.
[9] Perry, J., Deacon, R., Lumb, M. and Chanarin, I. (1980) Biochem. Biophys. Res. Commun. 97, 1329–1333.
[10] Barlowe, C.K. and Appling, D.R. (1988) Biochem. Biophys. Res. Commun. 157, 245–249.
[11] Rabinowitz, J.C. and Pricer, W.E. Jr (1962) J. Bio. Chem. 235, 2898–2902.
[12] Appling, D.R. and Rabinowitz, J.C. (1985) J. Biol. Chem. 260, 1248–1256.
[13] Lowry, O.H., Rosebrough, N.J., Lewis-Farr, A. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[14] Dunnett, C.W. (1955). J. Am. Stat. Assoc. 50, 1096–1121.