Biochemical Pharmacology, Vol. 38, No. 8, pp. 1353–1358, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

METHIONINE SYNTHASE ACTIVITIES IN MICE FOLLOWING ACUTE EXPOSURES TO ETHANOL AND NITROUS OXIDE

DONALD D. KOBLIN* and BARBARA W. TOMERSON

Department of Anesthesia, University of California, San Francisco, CA 94143; and Anesthesiology Service, Veterans Administration Medical Center, San Francisco, CA 94121, U.S.A.

(*Received* 24 *February* 1988; *accepted* 8 *September* 1988)

Abstract—Acute or chronic exposure to nitrous oxide or chronic exposure to ethanol decreases the activity of the vitamin B_{12}-dependent enzyme methionine synthase. To assess the combined effect of acute exposure to nitrous oxide and ethanol, mice were given an intraperitoneal injection of ethanol (3 g/kg) and exposed to an inspired mixture of 66% nitrous oxide and 34% oxygen for 4 hr. Methionine synthase activities in liver, kidney, and brain were measured immediately after exposure to nitrous oxide and at various times over a 4-day recovery period. Methionine synthase activities in liver and kidney returned to control levels 2–4 days following inactivation. In brain, a significant 16% decrease in methionine synthase activity remained after a 4-day recovery period. The acute administration of ethanol did not alter the magnitude of the inactivation induced by nitrous oxide nor the time course of recovery of methionine synthase activity following inactivation. Moreover, in mice that were not exposed to nitrous oxide, methionine synthase activity was not altered by the acute administration of ethanol alone or in combination with 0.4% atm isoflurane. Thus, in this animal model, an acute dose of ethanol does not alter methionine synthase activity nor does it enhance the inactivation produced by nitrous oxide.

Both ethanol and nitrous oxide can influence folate metabolism. Ethanol ingestion may interfere with the normal patterns of dietary folate intake, intestinal absorption of folate, transport of folate to tissues, and excretion of folate [1, 2]. Ethanol may also alter the activities of enzymes involved in one-carbon intermediate metabolism [1, 2]. Acute or chronic ingestion of ethanol may decrease serum folate levels and increase urinary and fecal excretion of folate in animals and humans [3–7]. Such alterations may cause the hematological disorders and other toxic effects observed in alcoholic patients [8, 9].

As with ethanol, nitrous oxide may impair the uptake of folate [10], increase the excretion of folate [11, 12], and deplete tissue stores of tetrahydrofolate [13–15]. These effects are probably due to oxidizing the cobalt atom of vitamin B_{12} by nitrous oxide [16], inactivating the enzyme methionine synthase [5-methyltetrahydrofolate: homocysteine methyltransferase (EC 2.1.1.13)] and preventing the conversion of methyltetrahydrofolate to tetrahydrofolate [17–19]. In rodents, a brief (30 min) exposure to subanesthetic concentrations of nitrous oxide inactivates >75% of hepatic methionine synthase [20, 21]. A similar but slower inactivation of methionine synthase occurs in human liver in patients who undergo routine surgical procedures and receive nitrous oxide as a component of their anesthetic [22].

The chronic administration of ethanol also decreases methionine synthase activity. Rats treated with ethanol for periods ranging from 3 days to 22 weeks exhibit a 28–47% decrease in methionine synthase activity in liver and kidney [23–25]. The influence of an acute dose of ethanol on methionine synthase activity has not been examined carefully.

Since both ethanol and nitrous oxide may disrupt folate metabolism and decrease methionine synthase activity, the possibility exists that the combined actions of these agents may have an especially deleterious effect. This combination of agents has clinical relevance since it is not unusual to administer nitrous oxide as a component of an anesthetic when an intoxicated patient or an alcoholic patient requires surgery. In the present study we employed the mouse as an animal model to examine the separate and combined influences of acute doses of ethanol and nitrous oxide on methionine synthase activity.

MATERIALS AND METHODS

Chemicals. [5-^{14}C]Methyltetrahydrofolate, barium salt (60 mCi/mmol), was obtained from the Amersham Corp. Anion exchange resin AG^{R} 1-X8 (200–400 mesh, chloride form) was from Bio-Rad Laboratories. Other biochemicals were purchased from the Sigma Chemical Co. Medical grade nitrous oxide and oxygen were from the Puritan-Bennett Corp. All other reagents were of the highest available purity.

Animals and treatments. These studies were approved by the Animal Studies Subcommittee at the Veterans Administration Medical Center, San Francisco. Male ICR mice (Simonsen Laboratories), initially weighing about 30 g, were provided Purina laboratory rodent chow and tap water *ad lib.* except during the 4-hr periods of exposure to nitrous oxide.

For a typical experiment, four mice were given a

* Correspondence: Dr. Donald D. Koblin, Anesthesiology Service (129), Veterans Administration Hospital, 4150 Clement St., San Francisco, CA 94121.

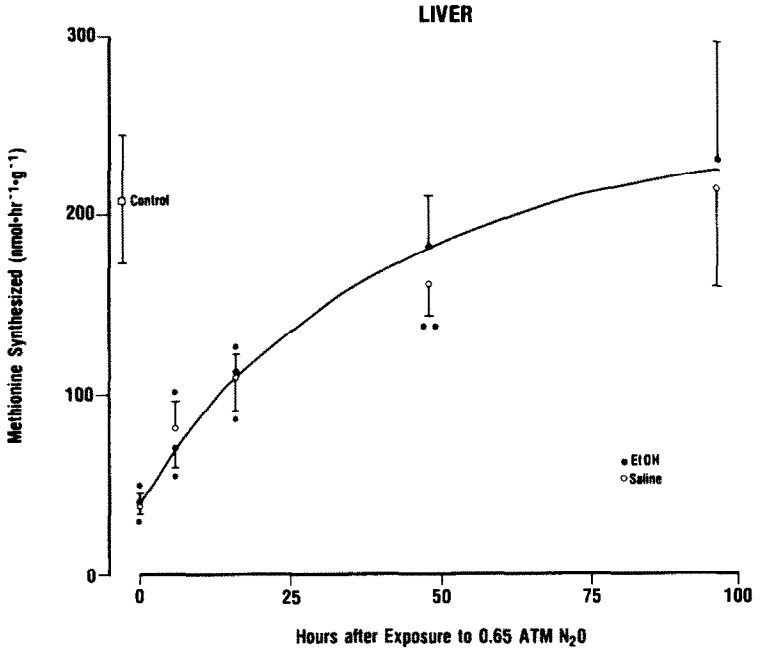

Fig. 1. Methionine synthase activities (expressed in nmol methionine produced·hr^{-1}·g^{-1}) in livers of mice at various times after exposure to 0.66 atm nitrous oxide for 4 hr. Exposed animals were injected intraperitoneally with either 3 g/kg ethanol (●) or saline (○) prior to administration of nitrous oxide. Control animals (□) were not injected and were not exposed to nitrous oxide. The control point represents the mean from sixteen mice. All other points represent the mean from eight animals. The error bars represent ±SD (only one side of the error bar is drawn for clarity). Nitrous oxide-treated animals were different from controls at significance levels of $^*P < 0.001$ or $^{**}P < 0.025$. At any given time point, no significant differences were detected between the saline-injected and ethanol-injected mice.

3.0 g/kg intraperitoneal injection of ethanol [20% (w/v) in 0.9% sodium chloride] and four mice were given an intraperitoneal injection of 0.9% sodium chloride (0.015 ml/g). Within 10 min after the injection of ethanol, the ethanol-injected and saline-injected mice were inserted into individual wire mesh cages and placed in a 20-liter stainless steel chamber. A mixture of nitrous oxide (5 L/min) and oxygen (2 L/min) was administered via an Ohio anesthesia machine for 10 min, and the gas flows were then decreased to 2 L/min for nitrous oxide and 1 L/min for oxygen for the duration of the 4-hr exposure. The stainless steel chamber was equipped with a fan that circulated chamber gases through a soda lime container to remove CO_2 [26, 27]. Chamber temperature remained near 23° but varied between 21.7 and 28.2°. Gas samples were removed from the chamber during the 4-hr exposure, and oxygen, nitrous oxide and carbon dioxide concentrations were measured with a Beckman model E-2 oxygen analyzer or a SARA mass spectrometer. Nitrous oxide concentrations averaged near 65%, oxygen concentrations ranged between 29 and 34%, and carbon dioxide was less than 1%. Exposures were carried out between 7:00 a.m. and 5:00 p.m.

Following the 4-hr exposure to 66% nitrous oxide, the chamber was flushed with 100% oxygen (approximately 10 L/min) for 2 min. At 0, 6, 16, 48, and 96 hr after the animals were removed from the nitrous oxide environment, mice were killed with 100% carbon dioxide. If the mice were not immediately killed, they were returned to their cages, allowed to breathe room air, and provided continuous access to rodent chow and water for the specified recovery time. Livers, kidneys, and whole brains were isolated from the animals, and the tissues were stored at −20 to −30°.

Three additional sets of experiments were performed in mice that were *not* exposed to nitrous oxide: (1) mice given a 3.0 g/kg intraperitoneal injection of ethanol; (2) mice given a 3.0 g/kg intraperitoneal injection of ethanol and exposed to 0.4% atm isoflurane ($CF_2H—O—CClH—CF_3$) for 4 hr; and (3) mice given an intraperitoneal injection of 0.9% NaCl (0.015 ml/g) and exposed to 0.4% atm isoflurane for 4 hr. Animals given only ethanol were killed 20–30 min after injection, and tissues were isolated for analysis of methionine synthase activity. Animals exposed to isoflurane were killed immediately after the 4-hr exposure. Isoflurane was administered in oxygen using a calibrated anesthetic vaporizer. Isoflurane concentrations were confirmed by mass spectrometry.

Sixteen mice served as controls to measure the normal values of methionine synthase activity in liver, kidney, and brain. These mice were obtained in the same shipment from the supplier but were not injected with ethanol, exposed to anesthetic, or placed in the exposure chamber.

Assays. Methionine synthase activity was determined as described previously [21]. Activity was expressed as nanomoles of methionine produced per

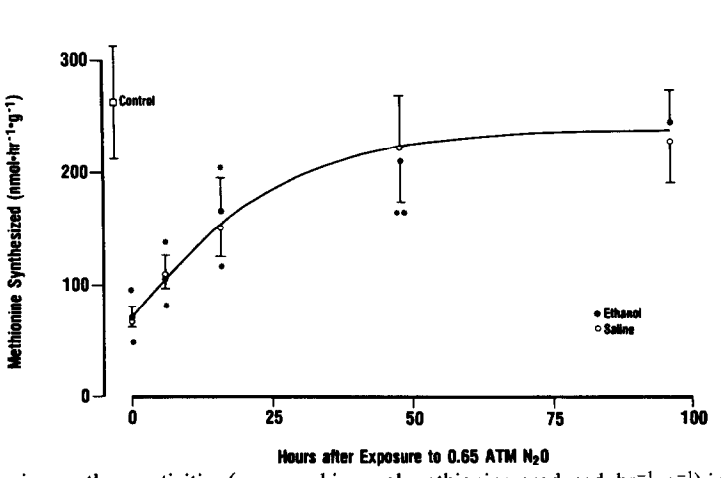

Fig. 2. Methionine synthase activities (expressed in nmol methionine produced·hr^{-1}·g^{-1}) in kidneys of mice at various times after exposure to 0.66 atm nitrous oxide for 4 hr. Exposed animals were injected intraperitoneally with either 3 g/kg ethanol (●) or saline (○) prior to administration of nitrous oxide. Control animals (□) were not injected and were not exposed to nitrous oxide. The control point represents the mean from sixteen mice. All other points represent the mean from eight animals. The error bars represent ±SD (only one side of the error bar is drawn for clarity). Nitrous oxide-treated animals were different from controls at significance levels of $^*P < 0.001$ or $^{**}P < 0.025$.

hour per gram of original tissue or as nanomoles methionine produced per hour per milligram of protein in the supernatant fraction. Protein content was determined by the method of Lowry *et al.* [28].

Statistical analysis. Statistical computations were performed with a one-way analysis of variance using the Neuman–Keuls test for multiple comparisons [29]. P values of <0.05 were considered statistically significant. Of the 120 mice examined in these experiments, none died upon injection of ethanol and/or exposure to anesthetic, and all values were included in the statistical analysis.

RESULTS

Methionine synthase activities in mice injected with either saline or ethanol and killed immediately after exposure to 66% nitrous oxide for 4 hr exhibited approximately an 82% decrease in liver (Fig. 1), a 75% decrease in kidney (Fig. 2), and a 58% decrease in brain (Fig. 3) when compared to the corresponding values in control mice that were not exposed to nitrous oxide. Methionine synthase activities in livers, kidneys, and brains of mice injected intraperitoneally with 3.0 g/kg of ethanol and killed immediately after exposure to 66% nitrous oxide for 4 hr did not differ significantly from those found in mice injected with saline and exposed to nitrous oxide (Figs. 1–3).

Some recovery of methionine synthase activity occurred within 6 hr after returning the saline- or ethanol-injected and nitrous oxide-exposed animals to an air environment. Sixteen hours after exposure to nitrous oxide, methionine synthase activities in liver, kidney, and brain averaged 53, 61, and 60% of the control values respectively (Figs. 1–3). After a 48-hr recovery period, methionine synthase activity remained significantly decreased in liver in the saline-injected mice (Fig. 1) and decreased in kidney in the ethanol-injected mice (Fig. 2). Activities in liver and kidney (Figs. 1 and 2) 96 hr following exposure to nitrous oxide did not differ from control values. In brain, a significant 16% decrease in methionine synthase activity was still evident in both the saline- and ethanol-injected mice 96 hr after exposure to nitrous oxide (Fig. 3). When the ethanol-injected animals were compared with the saline-injected animals, no differences in tissue methionine synthase activities were found at any given time point following exposure to nitrous oxide.

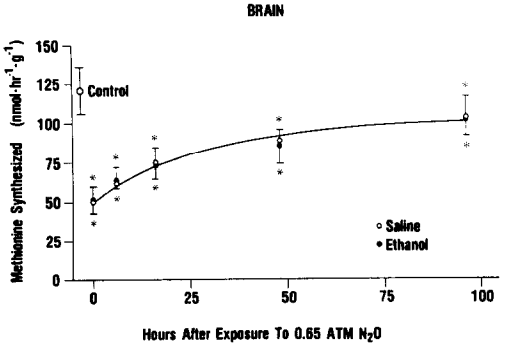

Fig. 3. Methionine synthase activities (expressed in nmol methionine produced·hr^{-1}·g^{-1}) in brains of mice at various times after exposure to 0.66 atm nitrous oxide for 4 hr. Exposed animals were injected intraperitoneally with either 3 g/kg ethanol (●) or saline (○) prior to administration of nitrous oxide. Control animals (□) were not injected and were not exposed to nitrous oxide. The control point represents the mean from sixteen mice. All other points represent the mean from eight animals. The error bars represent ±SD (only one side of the error bar is drawn for clarity). Nitrous oxide-treated animals were different from controls at a significance level of $^*P < 0.001$.

Table 1. Methionine synthase activities in mice administered ethanol and/or isoflurane

Treatment	Methionine synthesized (nmol·hr^{-1}·g^{-1})			N
	Liver	Kidney	Brain	
None (control)	209 ± 35.9	263 ± 50.1	122 ± 12.7	16
Ethanol (3 g/kg)	221 ± 54.7	258 ± 40.7	119 ± 19.2	8
Saline + isoflurane	183 ± 29.7	265 ± 47.0	126 ± 12.3	8
Ethanol + isoflurane	180 ± 37.4	226 ± 39.7	128 ± 11.8	8

Values are means ± SD. N is the number of animals examined. Exposure to isoflurane was 0.4% atm for 4 hr. Mice administered both ethanol and isoflurane were given a 3.0 g/kg intraperitoneal injection of ethanol and, within 10 min of injection, were exposed to 0.4% atm isoflurane for 4 hr.

Table 2. Ratios of methionine synthase activities

Tissue	Ratio (mg protein/g tissue)
Liver	118 ± 20.1
Kidney	83.9 ± 7.5
Brain	34.7 ± 2.2

The ratio for a given tissue in a given animal was calculated by taking the activity expressed in terms of nmol methionine produced·hr^{-1}·g^{-1} of original tissue and dividing by the activity expressed in terms of nmol methionine produced·hr^{-1}·(mg protein)$^{-1}$ in the supernatant fraction. Values are means ± SD obtained from 120 mice.

The influence of ethanol and isoflurane on methionine synthase activity was also examined. Treatment with intraperitoneal ethanol (3.0 g/kg), exposure to isoflurane (0.4%) for 4 hr, or a combination of these treatments did not alter significantly methionine synthase activities in liver, kidney, or brain when compared to control values (Table 1).

The ratios of methionine synthase activities when calculated on the basis of per gram of original tissue to that calculated on the basis of per milligram of protein in the supernatant fraction are summarized in Table 2. For any given tissue this ratio was consistent from animal to animal and did not vary with administration of ethanol, nitrous oxide, or isoflurane. However, this ratio varied among the different tissues with the value for liver a factor of 3.4 higher than the ratio for brain (Table 2).

DISCUSSION

The alterations in folate metabolism produced by either nitrous oxide or ethanol suggested that the combined use of these agents may be especially harmful. The major goals of the present experiments were: (1) to examine the effects of an acute dose of ethanol on methionine synthase activity in mouse liver, kidney, and brain; (2) to test whether an acute dose of ethanol enhanced the degree of inactivation produced by nitrous oxide; (3) to determine if the time course of recovery of methionine synthase activity following nitrous oxide-induced inactivation was delayed by an acute dose of ethanol; and (4) to characterize further the time course of recovery of methionine synthase activity in different tissues following exposure to nitrous oxide.

The acute dose of ethanol (3 g/kg) employed in these studies is in the range that increases urinary folate excretion in rats [5]. Following the injection of ethanol, all mice developed an unsteady gait and many transiently lost their righting reflex before being placed in the exposure chamber. Although ethanol levels were not measured in these particular animals, it is likely that peak ethanol levels were in the range of 300 mg/dl [30], i.e. a level near one that might occur with a binge drinking episode. Nitrous oxide (66% for 4 hr) was administered to these ethanol-treated animals in an attempt to mimic the not uncommon [31] clinical setting in which an intoxicated and traumatized individual requires surgery. Under such circumstances, nitrous oxide is often chosen as a component of the anesthetic because the patient may be in an unstable circulatory state and because alcohol decreases the requirement for more potent anesthetic agents [32].

Although the chronic administration of ethanol decreases methionine synthase activity in rat liver and kidney [23–25], the acute administration of ethanol did not alter methionine synthase activity in mouse liver, kidney, or brain (Table 1). Moreover, the magnitude of the nitrous oxide-induced inactivation of methionine synthase activity in mouse liver, kidney, or brain was not altered by the acute administration of ethanol, nor did ethanol treatment alter the time course of recovery following inactivation (Figs. 1–3).

The decrease (approximately 80%) in methionine synthase activity in mouse liver and kidney following a 4-hr exposure to 66% nitrous oxide (Figs. 1 and 2) is similar to the inactivation reported in previous studies for these tissues in rodents [13, 14, 20, 21, 33–36]. In mouse brain, a lesser degree (58%) of methionine synthase inactivation (Fig. 3) was produced by a 4-hr exposure to nitrous oxide than was found in liver or kidney. This lesser change in the brain is consistent with results from a previous study [21]. These differences between tissues are presumably due to the time course of inactivation, since exposures to nitrous oxide lasting 24 hr or longer can produce greater than a 90% inactivation in brain methionine synthase activity [14, 33, 37, 38].

The present experiments define more precisely the time course of recovery of hepatic methionine

synthase activity following inactivation by nitrous oxide, and they are the first studies to describe the rate of recovery of activity in kidney and brain. In liver, partial recovery of activity occurs as short as 6 hr after discontinuing the nitrous oxide. A small degree of enzyme inactivation may be present in liver 2 days after nitrous oxide exposure, but enzyme activity returned to control values by 4 days post-exposure (Fig. 1) in agreement with previous work [21]. Recovery of hepatic methionine synthase activity in the mouse may be slightly faster than in the rat, since activity in rat liver remains depressed after a 3-day recovery period [13, 33, 35, 36]. The time course of recovery of enzyme activity in mouse kidney was similar to that in liver, with recovery being complete 4 days after exposure to nitrous oxide (Fig. 2). However, methionine synthase activity was depressed significantly at 2 days of recovery in the kidneys of ethanol-injected but not saline-injected animals (Fig. 2), whereas the opposite effect was found in livers (Fig. 1). Although the magnitude of nitrous oxide-induced enzyme inactivation in the brain was less than that in liver or kidney, recovery of methionine synthase activity was slower in brain than in liver or kidney, remaining significantly depressed following 4 days of recovery in both ethanol-injected and saline-injected animals (Fig. 3).

Isoflurane was employed as an anesthetic control in the present experiments to ensure that the effects of nitrous oxide on methionine synthase activity (with and without ethanol) were not simply due to physiological alterations caused by subanesthetic concentrations. In terms of ability to abolish the righting reflex in mice, the concentration of isoflurane used (0.4% atm) is slightly more potent than 66% nitrous oxide [39]. However, neither isoflurane alone or in combination with ethanol altered methionine synthase activity in any of the three tissues examined (Table 1).

In previous investigations, methionine synthase activities have been reported either on the basis of fresh tissue weight or on the basis of milligram protein in the supernatant fraction but not both. This difference in reporting techniques makes it difficult to compare how absolute activities vary among species and from one laboratory to the next. For example, in one study [40] comparisons among literature values for methionine synthase activities in human liver and kidney were made by assuming that 100 mg of protein in the supernatant fraction would correspond to 1 g of fresh tissue. This assumed ratio is very nearly the average of the ratios we measured in mouse liver (118) and kidney (83.9) (Table 2). However, the ratio of milligrams protein to grams tissue was markedly lower in the brain compared to liver or kidney (Table 2).

Although the acute administration of ethanol did not enhance the nitrous oxide-induced inactivation of methionine synthase in these experiments, the possibility remains that the combination of these agents may alter folate status to a greater extent than each individual agent. For instance, combined nitrous oxide and ethanol treatment may have had a synergistic effect in increasing urinary and fecal excretion of folate or altering tissue levels of folates, parameters that were not determined in the present studies. In addition, the inability to find an enhanced effect in the mouse does not preclude the possibility that other species may be more susceptible to the combined acute administration of these drugs. Specific activities of hepatic methionine synthase are similar in rats, humans, and monkeys, but marked species differences exist in hepatic tetrahydrofolate concentrations, with levels in mice approximately 6-fold greater than those of humans [41]. Furthermore, animals [23, 42] or humans [12, 43] with a subclinical vitamin B_{12} or folate deficiency or in a poor nutritional state may be more susceptible to ethanol and nitrous oxide. Finally, it remains to be determined whether animals chronically treated with ethanol have an increased susceptibility to the toxic effects of nitrous oxide.

Acknowledgements—This research was supported by a Regional Advisory Group Grant from the Veterans Administration, by a Merit Review Grant from the Veterans Administration, by a grant from the Academic Senate of the University of California at San Francisco, by NIA Grant PO1 AG3104, and by the Anesthesia Research Foundation. We thank Dr. Edmond I. Eger, II, for reading the manuscript.

REFERENCES

1. Weir DG, McGing PG and Scott JM, Folate metabolism, the enterohepatic circulation and alcohol. *Biochem Pharmacol* **34**: 1–7, 1985.
2. Hillman RS and Steinberg SE, The effects of alcohol on folate metabolism. *Annu Rev Med* **33**: 345–354, 1982.
3. Tamura T and Halsted CH, Folate turnover in chronically alcoholic monkeys. *J Lab Clin Med* **101**: 623–628, 1983.
4. Russell RM, Rosenberg IH, Wilson PD, Iber FL, Oaks EB, Giovetti AC, Otradovec CL, Karwoski PA and Press AW, Increased urinary excretion and prolonged turnover time of folic acid during ethanol ingestion. *Am J Clin Nutr* **38**: 64–70, 1983.
5. McMartin KE, Collins TD, Shiao CQ, Vidrine L and Redetzki HM, Study of dose-dependence and urinary folate excretion produced by ethanol in humans and rats. *Alcoholism: Clin Exp Res* **10**: 419–424, 1986.
6. Eisenga BH and McMartin KE, The effect of acute ethanol administration on the clearance of ^{3}H-Pteglu by the rat kidney. *Nutr Res* 7: 1051–1060, 1987.
7. Blocker DE and Thenen SW, Intestinal absorption, liver uptake, and excretion of ^{3}H-folic acid in folic acid-deficient, alcohol-consuming nonhuman primates. *Am J Clin Nutr* **46**: 503–510, 1987.
8. Kanazawa S and Herbert V, Detection of folate deficiency in alcoholism using the peripheral blood lymphocyte deoxyuridine suppression test. *J Nutr Sci Vitaminol* **32**: 251–257, 1986.
9. Lindenbaum J, Hematologic complications of alcohol abuse. *Semin Liver Dis* 7: 169–181, 1987.
10. Perry J, Deacon R, Lumb M and Chanarin I, Impaired formylation and uptake of tetrahydrofolate by rat small gut following cobalamin inactivation. *Biochim Biophys Acta* **923**: 286–290, 1987.
11. Lumb M, Perry J, Deacon R and Chanarin I, Urinary folate loss following inactivation of vitamin B_{12} by nitrous oxide in rats. *Br J Haematol* **51**: 235–242, 1982.
12. Amos RJ, Amess JAL, Hinds CJ and Mollin DL, Investigations into the effect of nitrous oxide anaesthesia on folate metabolism in patients receiving intensive care. *Chemioterapia* **4**: 393–399, 1985.
13. Black KA and Tephly TR, Effects of nitrous oxide and methotrexate administration on hepatic methionine

synthetase and dihydrofolate reductase activities, hepatic folates, and formate oxidation in rats. *Mol Pharmacol* **23**: 724–730, 1983.
14. Wilson SD and Horne DW, Effect of nitrous oxide inactivation of vitamin B_{12} on the levels of folate coenzymes in rat bone marrow, kidney, brain, and liver. *Arch Biochem Biophys* **244**: 248–253, 1986.
15. Makar AB and Tephly TR, The role of formate and *S*-adenosylmethionine in the reversal of nitrous oxide inhibition of formate oxidation in the rat. *Mol Pharmacol* **32**: 309–314, 1987.
16. Banks RGS, Henderson RJ and Pratt JM, Reactions of gases in solution. Part III. Some reactions of nitrous oxide with transition-metal complexes. *J Chem Soc (A)*: 2886–2888, 1968.
17. Shane B and Stokstad ELR, Vitamin B_{12}-folate interrelationships. *Annu Rev Nutr* **5**: 115–145, 1985.
18. Chanarin I, Deacon R, Lumb M and Perry J, Cobalamin-folate interrelations: A critical review. *Blood* **66**: 479–489, 1985.
19. Nunn JF, Clinical aspects of the interaction between nitrous oxide and vitamin B_{12}. *Br J Anaesth* **59**: 3–13, 1987.
20. Deacon R, Lumb M, Perry J, Chanarin I, Minty B, Halsey MJ and Nunn JF, Selective inactivation of vitamin B_{12} in rats by nitrous oxide. *Lancet* **2**: 1023–1024, 1978.
21. Koblin DD, Watson JE, Deady JE, Stokstad ELR and Eger EI II, Inactivation of methionine synthetase by nitrous oxide in mice. *Anesthesiology* **54**: 318–324, 1981.
22. Koblin DD, Waskell L, Watson JE, Stokstad ELR and Eger EI II, Nitrous oxide inactivates methionine synthetase in human liver. *Anesth Analg* **61**: 75–78, 1982.
23. Finkelstein JD, Cello JP and Kyle WE, Ethanol-induced changes in methionine metabolism in rat liver. *Biochem Biophys Res Commun* **61**: 525–531, 1974.
24. Barak AJ, Beckenhauer HC and Tuma DJ, Ethanol feeding inhibits the activity of hepatic N^5-methyltetrahydrofolate-homocysteine methyltransferase in the rat. *IRCS J Med Sci* **13**: 760–761, 1985.
25. Molloy AM, Scott JM and Weir DG, Alcohol induced methyl group wastage as a possible cause of fatty liver. In: *Chemistry and Biology of Pteridines* (Eds. Cooper BA and Whitehead VM), pp. 505–508. Walter de Gruyter, New York, 1986.
26. Halsey MJ, Eger EI II, Kent DW and Warne PJ, High-pressure studies of anesthesia. In: *Progress in Anesthesiology* (Ed. Fink BR), Vol. 1, pp. 353–361. Raven Press, New York, 1975.
27. Koblin DD, Dong DE and Eger EI II, Tolerance of mice to nitrous oxide. *J Pharmacol Exp Ther* **211**: 317–325, 1979.
28. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
29. Zar JH, *Biostatistical Analysis*, 2nd Edn, pp. 190–191. Prentice-Hall, Englewood Cliffs, 1984.
30. Koblin DD, Deady JE, Dong DE and Eger EI II, Mice tolerant to nitrous oxide are also tolerant to alcohol. *J Pharmacol Exp Ther* **213**: 309–312, 1980.
31. Soderstrom CA and Cowley RA, A national alcohol and trauma center survey. *Arch Surg* **122**: 1067–1071, 1987.
32. Nunn JF, Chanarin I, Tanner AG and Owen ERTC, Megaloblastic bone marrow changes after repeated nitrous oxide anaesthesia. *Br J Anaesth* **58**: 1469–1470, 1986.
33. Deacon R, Lumb M, Perry J, Chanarin I, Minty B, Halsey M and Nunn J, Inactivation of methionine synthase by nitrous oxide. *Eur J Biochem* **104**: 419–422, 1980.
34. Eells JT, Black KA, Makar AB, Tedford CE and Tephly TR, The regulation of one-carbon oxidation in the rat by nitrous oxide and methionine. *Arch Biochem Biophys* **219**: 316–326, 1982.
35. Kondo H, Osborne ML, Kolhouse JF, Binder MJ, Podell ER, Utley CS, Abrams RS and Allen RH, Nitrous oxide has multiple deleterious effects on cobalamin metabolism and causes decreases in activities of both mammalian cobalamin-dependent enzymes in rats. *J Clin Invest* **67**: 1270–1283, 1981.
36. Baden JM, Serra M and Mazze RI, Inhibition of fetal methionine synthetase activity by nitrous oxide. *Br J Anaesth* **56**: 523–526, 1984.
37. Hakim AM, Cooper BA, Rosenblatt DS and Pappius HM, Local cerebral glucose utilization in two models of B_{12} deficiency. *J Neurochem* **40**: 1155–1160, 1983.
38. Xue G, Snoswell AM and Runciman WB, Perturbation of methionine metabolism in sheep with nitrous oxide-induced inactivation of cobalamin. *Biochem Int* **12**: 61–69, 1986.
39. Koblin DD and Eger EI II, How do inhaled anesthetics work? In: *Anesthesia* (Ed. Miller RD), 2nd Edn, pp. 581–623. Churchill Livingstone, New York, NY, 1986.
40. Baumgartner ER, Stokstad ELR, Wick H, Watson JE and Kusano G, Comparison of folic acid coenzyme distribution patterns in patients with methylenetetrahydrofolate reductase and methionine synthetase deficiencies. *Pediatr Res* **19**: 1288–1292, 1985.
41. Johlin FC, Fortman CS, Nghiem DD and Tephly TR, Studies on the role of folic acid and folate-dependent enzymes in human methanol poisoning. *Mol Pharmacol* **31**: 557–561, 1987.
42. Van De List C, Combs M and Schilling RF, Nitrous oxide and vitamin B_{12} deficiency interact adversely on rat growth. *J Lab Clin Med* **108**: 346–348, 1986.
43. Schilling RF, Is nitrous oxide a dangerous anesthetic for vitamin B_{12}-deficient subjects? *JAMA* **255**: 1605–1606, 1986.