Review Article

Antivitamin action of nitrous oxide in OMF surgery—a narrative review

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Background and Objective: Nitrous oxide was introduced in 1844 as an inhaled anesthetic in dentistry. It is usually well tolerated, but its inhibition of vitamin B12 (cobalamin) function was noted in 1956. Clinical ramifications are summarized.

Methods: Via PubMed, English language papers were selected from the MEDLINE database from 1956 to 2022.

Key Content and Findings: When patients inhale anesthetic concentrations of nitrous oxide, the agent covalently inactivates the vitamin B12-dependent enzyme methionine synthase. Nitrous oxide is a strong oxidant molecule, known for instance to support candle flames. The cobalt atom of the vitamin is in a univalent state in the active enzyme and is a chemically strong reductant. A redox reaction of the cobalt ion with the oxide generates hydroxyl radicals that chemically attack both the B12 cofactor and the polypeptide chain of the enzyme. Inactivation of the synthase pathologically elevates plasma levels of the amino acid homocysteine, normally a precursor of methionine. Recovery of enzyme function requires biosynthesis of fresh enzyme molecules, starting from vitamin B12 and free amino acids.

Conclusions: Though a temporary and partial defect in methionine synthase activity does not necessarily result in harm, sensitive patients may experience neuropathy and problems related to impaired DNA synthesis. Risk factors that increase susceptibility to N_2O toxicity include prolonged or frequent N_2O exposure; concurrent methotrexate therapy; genetic deficiency of methylene-tetrahydrofolate reductase; pernicious anemia; and poor nutrition. Nutritional supplements, including folinic acid, can prevent or relieve N_2O toxicity, but their clinical reliability is uncertain.

Keywords: Cobalamin; folic acid; homocysteine; methionine synthase; pyridoxine

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Introduction

Background

As an aid to dentistry, nitrous oxide was the first inhaled anesthetic to reach public attention. Now, many, perhaps most, inhaled anesthetics for OMF surgery include the gas. Its antivitamin action is generally disregarded. Accordingly, the primary objective of this review is to illuminate this often subtle but detectable toxicity.

The gas was discovered in 1772 in bubbles arising

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Table 1 Search strategy summary

Items	Specification
Date of search	1 February 2022 to 8 August 2022
Databases and other sources searched	MEDLINE
Search terms used	Cobalamin; DNA; folinic acid; homocysteine; methionine; myelin; nitrous, oxide; pernicious anemia; pyridoxal; spongiform; tetrahydrofolic acid; vitamin B12
Timeframe	1956 to 2022
Inclusion criteria	Primary reports favored; English language
Selection process	Reviewer discretion

from the action of aqueous nitric acid on metals. In 1799, Humphry Davy found the pure gas to be nonirritating and intoxicating (1,2). He staged popular demonstrations at the Royal Institution in London, and the substance became known as "laughing gas" (1).

Medical student Gardner Quincy Colton staged laughing gas shows in America (3,4). After witnessing a Colton show in 1844, American dentist Horace Wells proposed nitrous oxide for painless tooth extractions (5). Wells failed to convince witnesses at the Harvard Medical School that nitrous is an effective anesthetic (5), but his partner William T.G. Morton successfully introduced relatively potent diethyl ether vapor there (6). The landmark Morton demonstration was for an OMF surgery, the excision of a jaw tumor, on October 16, 1846. Despite the triumph of Morton on Ether Day, Colton clung to nitrous and became the busiest painless dentist in Manhattan (4).

The last diethyl ether anesthetic at Harvard was in 1978, but nitrous oxide is a frequent anesthetic agent today. However, the gas interferes with the function of vitamin B12. It covalently inactivates the enzyme methionine synthase, one of the two important B12 enzymes in humans.

Rationale and knowledge gap

We consider the mechanism and the clinical ramifications of the antivitamin phenomenon.

Objective

We consider situations in which nitrous oxide toxicity may be appreciable.

We present the following article in accordance with the Narrative Review reporting checklist (available at https://joma.amegroups.com/article/view/10.21037/joma-22-21/rc).

Methods

By means of the PubMed search engine of the United States National Library of Medicine, the MEDLINE database of references and abstracts on life sciences and biomedical topics was searched for information in the English language from 1956 to 2022 (*Table 1*). Full articles were accessed via the Countway Library of the Harvard Medical School. SANRA criteria were guidelines.

Findings

Antivitamin action

Evidence of antagonism of vitamin B12 appeared in 1956 (7). Tetanus victims requiring mechanical ventilation, sedation, and analgesia were ventilated with 50% N_2O for several days, whereupon peripheral blood smears became marked by megaloblasts (7). Megaloblasts are large, nucleated precursors of red corpuscles. They do not normally circulate but are particularly prominent in so-called pernicious anemia (7). Pernicious anemia was recognized in the 1800s as an anemia that was usually fatal within three years of its symptoms. The disease led to the life-saving discovery of vitamin B12 in the 1900s. Accordingly, the megaloblastosis of nitrous oxide exposure pointed to the deficient function of vitamin B12 (7).

Methionine synthase

There are two vitamin B12-dependent enzymes in humans. They are methionine synthase (*Figure 1*) and methylmalonyl-CoA mutase. The first of these is covalently

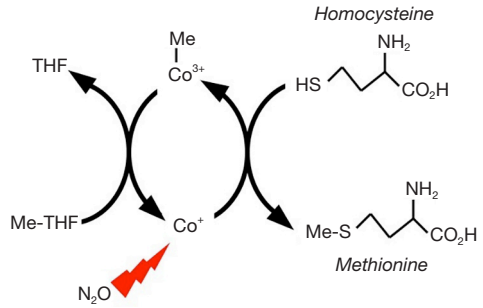

Figure 1 The two steps by which the methionine synthase reaction proceeds. A cobalt ion of the prosthetic B12 cofactor carries a methyl group (Me; CH_3-) from Me-THF to homocysteine. The univalent cobalt ion of the demethylated enzyme is a strong reductant and is intercepted by N_2O. The enzyme becomes covalently inactivated by a free radical generated in the reaction of N_2O with the cobalt ion. Me-THF, methyl-tetrahydrofolate.

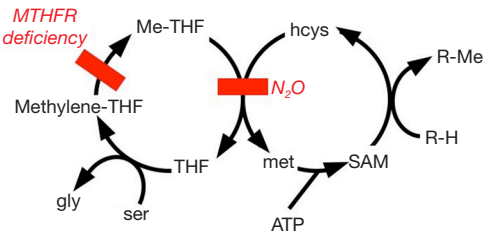

Figure 2 Coupling of the methionine cycle to the THF cycle. The amino acid ser yields a one-carbon group to tetrahydrofolate and becomes the smaller amino acid gly. MTHFR converts methylene-THF to Me-THF, and met synthase carries the methyl group to hcys, thus forming methionine. Methionine reacts enzymatically with ATP to afford SAM. SAM is the substrate for various methylation enzymes that convert R-H to R-Me. R designates various groups of atoms. Examples of R-Me include choline, epinephrine, and O-methylated catecholamines. The other product of the methylation reaction is converted to homocysteine. Methionine and homocysteine are not consumed when the cycle is functional. However, the cyclic process can be blocked when MTHFR is genetically deficient or when methionine synthase is inactivated by N_2O. THF, tetrahydrofolate; ser, serine; gly, glycine; MTHFR, methylenetetrahydrofolate reductase; Me-THF, methyl-tetrahydrofolate; met, methionine; hcys, homocysteine; SAM, S-adenosylmethionine.

inactivated by N_2O. The synthase reaction proceeds according to Equation [1].

$$\text{Methyltetrahydrofolate} + \text{homocysteine} \rightarrow \text{tetrahydrofolate} + \text{methionine} \quad [1]$$

Methionine is one of the 20 amino acids that commonly comprise protein. It is an "essential" amino acid, required in the diet. Why then is a methionine-synthesizing enzyme important in human metabolism? Methionine is an S-methyl compound and is an important source of one-carbon (methyl) groups in biosynthesis (*Figure 2*). Many methylations in metabolism require methionine to be consumed and then rebuilt by methionine synthase in the so-called methionine cycle. For instance, norepinephrine is methylated to epinephrine by an N-methyltransferase, and catecholamines are inactivated upon methylation by an O-methyltransferase. Other examples of methyl groups arising from methionine are those of acetylcholine and choline-containing lipids. The lecithin of bile is a choline-containing lipid (which is historically why the word acetylcholine sounds like cholecystectomy). Another choline-derived lipid is sphingomyelin, a major component of myelin sheaths of nerves. A methylation that indirectly involves methionine synthase is the process by which uracil becomes the thymine of DNA (*Figure 3*).

The vitamin B12 molecule contains a cobalt ion and is thus also known as cobalamin. In vitamin pills, the molecule is stabilized by a functionally inactive cyanide group and is also called cyanocobalamin. The cobalt atom of cyanocobalamin is in an oxidized trivalent state (Co^{3+}) and is not further oxidized by nitrous oxide. However, in the catalytic active site of methionine synthase, the cyanide-free cobalt functions in the unstable Co^{1+} state (*Figure 1*). Unshielded by the protein, highly reactive univalent cobalt can reduce water to hydrogen gas. At anesthetic concentrations, N_2O can gain access to the reactive cobalt atom. The N_2O is reduced by one-electron to a hydroxyl free radical (HO·) that then inactivates a vital part of the enzyme (8). The hydroxyl radical arises according to Equation [2].

$$Co^{1+} + N_2O + H^+ \rightarrow Co^{2+} + N_2 + HO \quad [2]$$

The damaged enzyme cannot be repaired, and recovery requires synthesis of new enzyme molecules from fresh amino acids, a process with a half-time of days in humans (8,9). The rate of inactivation of the enzyme is not precisely known, and the process might not be monophasic *in vivo*. Probably because of high metabolic rates, rodents are relatively sensitive. Liver biopsies in humans show that about half of the enzyme is inactivated by 50–70% nitrous in a few hours (9).

Methylmalonyl-CoA mutase is involved in the catabolism

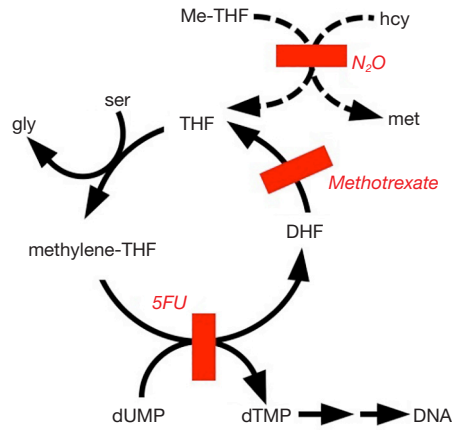

Figure 3 Cycling of tetrahydrofolate in the biosynthesis of DNA. Inhibition of tetrahydrofolate formation occurs upon inactivation of methionine synthase by N_2O and also by inhibition of dihydrofolate reductase by methotrexate. Those inhibitors, as well as 5FU, inhibit the methylation of dUMP to dTMP for the synthesis of DNA. 5FU, 5-fluorouracil; Dump, deoxyuridine; dTMP, thymidine.

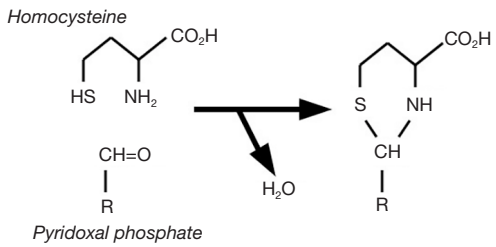

Figure 4 Spontaneous reaction of homocysteine with aldehyde groups. Biomolecules with aldehyde groups (-CH=O) include pyridoxal phosphate, a cofactor for many enzymes of amino acid metabolism. Here, the pyridoxal phosphate molecule is shown simply as R-CH=O. Cyclization of pyridoxal phosphate with homocysteine depletes pyridoxal phosphate levels and yields a six-membered cyclic adduct that competitively inhibits pyridoxal phosphate-dependent enzymes.

of fatty acids and amino acids that give rise to propionic acid, CH_3-CH_2-COOH. The mutase does not react directly with N_2O. However, prolonged exposures to N_2O may indirectly reduce the mutase level since cobalamin is consumed in methionine synthase reaction with N_2O. The cofactor for the mutase is a form of vitamin B12 known as adenosylcobalamin, and it is not known if N_2O affects the adenosylation of cobalamin.

Consequences of inactivation of methionine synthase

There are at least three consequences of the inactivation of methionine synthase. They are impaired DNA synthesis, neuropathy, and elevation of plasma homocysteine.

DNA synthesis is inhibited because methionine synthase is involved in the incorporation of thymine into DNA (*Figure 3*). Dietary thymine is poorly utilized in the synthesis of DNA. Instead, a deoxyuridine nucleotide is methylated to a thymidine one. Impaired DNA synthesis after N_2O exposure has been demonstrated in humans by means of an *ex-vivo* cellular assay known as the deoxyuridine suppression test, in which deoxyuridine inhibits incorporation of radiolabeled thymidine into DNA (10). Other evidence includes cases of megaloblastosis and macrocytic anemia (11).

As in the case of untreated pernicious anemia, N_2O has been associated with cases of demyelination of the spinal cord (11-17). N_2O has been associated with other neuropathies (18-21) and psychiatric problems (22-27) in patients at increased risk of toxicity. Demyelination may arise because methionine synthase provides methyl groups for the sphingomyelin lipid molecule (*Figure 2*). The enzyme also provides methyl groups for acetylcholine, epinephrine, and O-methyl catecholamines.

The *in vitro* assay of methionine synthase is cumbersome and is best suited to biopsy specimens of solid tissues. Accordingly, the kinetics of enzyme inactivation and regeneration are known only roughly in humans. However, acute metabolic action of nitrous oxide is readily detected as an increase in plasma levels of homocysteine (28-34). Homocysteine is the precursor of methionine in the synthase reaction (*Figure 1*).

Though homocysteine is an essential metabolic intermediate, its elevated concentration is toxic. For one thing, the molecule spontaneously forms a stable covalent derivative of pyridoxal phosphate, the enzymatically active form of the B6 vitamin pyridoxine (*Figure 4*) (35,36). Adduct formation depletes pyridoxal phosphate (the enzyme-cofactor form of pyridoxine), and the adduct is an inhibitor of pyridoxal phosphate dependent enzymes. These include serine hydroxymethyltransferase, which converts tetrahydrofolate into methylene-tetrahydrofolate (*Figures 2,3*). Consequently, impaired function of vitamin B12 disrupts function of the vitamins B6 (pyridoxine) and B9 (folic acid).

Another mechanism of homocysteine toxicity involves its metabolism to a chemically reactive product. The ATP-utilizing synthetase that normally attaches methionine to tRNA also attaches homocysteine to tRNA. Methionyl-

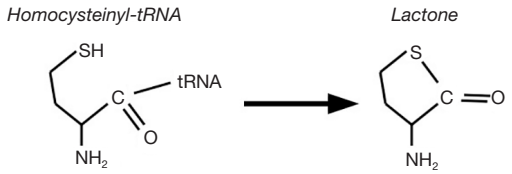

Figure 5 Spontaneous cyclization of homocysteinyl-tRNA to a chemically reactive thiolactone. Unlike methionine, homocysteine is not incorporated into proteins. However, homocysteine is a substrate for the enzyme that ordinarily uses ATP to attach methionine to transfer-RNA. Methionyl-tRNA is used in ribosomal protein synthesis. Homocysteinyl-tRNA is unstable and cyclizes into a five-membered ring called a thiolactone. The lactone can spontaneously attach to various cellular constituents. In particular, it covalently inactivates the active site of lysyl oxidase, an enzyme that cross-links collagen and elastin fibers of blood vessels and other connective tissues.

tRNA is used for protein synthesis, but homocysteinyl-tRNA is unstable (*Figure 5*). It yields a cyclic thioester known as homocysteine thiolactone (37). The thiolactone can nonspecifically attach to cellular constituents, and it specifically blocks the active site of lysyl oxidase, an enzyme involved in the cross-linking of strands of collagen and elastin in blood vessels and other connective tissues (38).

The inactivation of lysyl oxidase may contribute to the atherogenic action of homocysteine (39). However, that and prothrombotic actions might involve reactions of the thiolactone and/or homocysteine with other targets (20,39-42). For instance, they affect vascular endothelium. In particular, they inhibit endothelial production of nitric oxide (NO), a two-atom gas molecule that is not to be confused with the three-atom anesthetic nitrous oxide (N_2O). The subject of a Nobel Prize in 1997, NO inhibits platelet adhesion and aggregation, and it dilates blood vessels. In addition to inhibiting endothelial NO synthesis, sulfur compounds such as homocysteine react directly with and thus scavenge NO (28).

Cardiovascular complications have been ascribed to chronic hyperhomocysteinemia (39,40). However, the ENIGMA-II study demonstrated the long-term safety of one-time nitrous oxide administration as part of the anesthetic regimen in noncardiac surgical patients with known or suspected coronary artery disease (43,44).

Risk factors for N_2O toxicity

Serious metabolic problems are rarely seen when N_2O is used as an anesthetic. Risk factors for toxicity include prolonged exposure (24,45,46); methotrexate and related drug therapy (47-49); genetic problems such as deficiency of methylene-tetrahydrofolate reductase; pernicious anemia (7,11); and, presumably, dietary deficiency of vitamins.

Prolonged exposure to N_2O can include recreational abuse of the intoxicating drug (24) as well as occupational exposure (45). Recreational exposure can involve small cylinders of the gas known as whippits or whippets. These are intended for use in the whipping of cream for culinary purposes. The agent is discharged into party balloons in decompression for inhalation. Occupational exposure is most likely to occur near patients receiving the gas by loose-fitting masks. The inactivation of methionine synthase persists following discontinuation of an anesthetic, so toxicity can arise from either lengthy anesthetics or else repetitive exposure (46).

Methotrexate inhibits dihydrofolate reductase, and N_2O inactivates methionine synthase. Both enzymes produce tetrahydrofolate, so the combined inhibitors can profoundly impair the availability of tetrahydrofolate to participate in the synthesis of DNA. In laboratory experiments with rodents, N_2O is easily demonstrated to convert nonlethal doses of methotrexate into lethal doses (47). Potentiation of methotrexate by N_2O has also been encountered in humans (48,49).

Any genetic defect in folate or vitamin B12 metabolism might predispose to N_2O toxicity. One example is deficiency of methylene-tetrahydrofolate reductase (12,50-53). As exemplified by pernicious anemia, acquired problems in B12 or folate status can also facilitate N_2O toxicity (54). Nutritional depletion of protein and/or vitamins would also increase the likelihood of metabolic complications of N_2O (55-57). Preoperative supplementation of vitamin B12 reduces postoperative elevation of homocysteine (35,58), but the effect is variable (59).

Treatment/rescue

A lesson from pernicious anemia was that vitamin B12 deficiency can be palliated by nutritional agents. For instance, the anemia of early pernicious anemia could be masked by doses of folic acid. Unfortunately, the neuropathy of the disease proceeded in spite of folate supplementation. One strategy has been to provide supplemental tetrahydrofolate. However, that molecule is significantly unstable in the presence of oxygen. Accordingly, tetrahydrofolate is given in the form of a

relatively stable derivative known as folinic acid (60,61). Chemically, that is N-formyltetrahydrofolate. The molecule was discovered in 1948 as an essential growth factor acid for the bacterium *Leuconostoc citrovorum*, and it has thus been called citrovorum factor and also leucovorin. It was clinically introduced for rescue from methotrexate. It permits some DNA synthesis in the presence of inhibitors of dihydrofolate reductase or methionine synthase (*Figure 3*). However, full rescue would require its recycling in the body, and the recycling is inhibited by the presence of the mentioned enzyme inhibitors.

Exogenous thymine base is poorly utilized for DNA synthesis (62-65), but the nucleoside thymidine merits investigation as a rescue agent in view of benefit seen in pernicious anemia (66). Since sphingomyelin of myelin is a choline ester, it would be of interest to examine dietary choline for prevention of N_2O-induced demyelination (67).

Dietary supplementation with exogenous methionine and betaine (N,N,N-trimethylglycine) can palliate errors in cobalamin metabolism (68). The most common case is cobalamin C disorder, in which dietary cyanocobalamin and other forms of vitamin B12 are not converted to the enzymatically active form for methionine synthetase (nor that for methylmalonyl-CoA mutase). When a patient with early onset of the disorder consistently took methionine and betaine, methionine levels were raised to normal levels (69). Normalizing methionine levels may prevent CNS sequelae associated with cobalamin C disorder, including subacute combined degeneration (SCD) of the spinal cord (69). However, these findings have not been well studied in humans. Methionine supplementation is protective against SCD in monkeys and pigs exposed to nitrous oxide (70,71).

Because the cobalamin cofactor is covalently consumed in the reaction of methionine synthase with N_2O, administration of vitamin B12 is plausibly beneficial before or after administration of N_2O. Vitamin B12 is clearly indicated if its deficiency is clinically apparent.

Theoretical considerations

In view of the strong oxidation-reduction potential of the Co^{1+} center of methionine synthase, other oxidants in addition to N_2O might also react with the enzyme. The one drug in addition to N_2O that is known to inactivate methionine synthase is chloroform (72-75). Inactivation of methionine synthesis by chloroform was demonstrated in a B12-dependent *E. coli* strain after chloroform and related molecules were noted to block B12-dependent methane biosynthesis in the rumen-dwelling bacteria of cattle (76-78). It is intriguing to wonder if N_2O or chloroform might have exploitable antibiotic activity against pathogenic B12-dependent microbes. For instance, malaria parasites carry a B12-dependent methionine synthase (79). Presently, there are no examples of nitrous oxide as an antimicrobial drug, and its inhalation in anesthetic doses precludes hyperoxia as an anti-anaerobe strategy (46,80).

N_2O is selectively toxic to dividing neoplastic cells (46,78-85). In view of the permeability of skin to inhaled anesthetics (86), it would be interesting to evaluate the gas for topical therapy of cutaneous lesions.

Conclusions

Inhaled N_2O is a useful and generally safe anesthetic agent. However, it antagonizes the function of vitamin B12, a factor that participates in DNA synthesis, nerve myelination, and homocysteine scavenging. Accordingly, it is prudent to avoid prolonged or repetitive N_2O exposures to anesthetic/sedative doses. Alternative anesthetic agents should be considered in cases of neuropathy and in cases of relevant metabolic impairments such as those of concurrent methotrexate therapy; genetic deficiency of methylene-tetrahydrofolate reductase; pernicious anemia; and poor nutrition. However, neither routine screening for vitamin B12 status, nor supplementation with vitamin B12, is likely to have clinical value for one-time anesthetic exposures of a few hours or less.

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Footnote

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Conflicts of Interest: Both authors have completed the

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