

## CLINICAL ASPECTS OF THE INTERACTION BETWEEN NITROUS OXIDE AND VITAMIN B<sub>12</sub>

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Vitamin B<sub>12</sub> is a bound coenzyme of methionine synthase and has a tetrapyrrole ring with monovalent cobalt at the centre. The cobalt functions as a methyl carrier in the transmethylation reaction shown in figure 1. In 1968, Banks, Henderson and Pratt reported that nitrous oxide reacted *in vitro* with vitamin B<sub>12</sub>, converting the cobalt from the monovalent form (Cob(I)alamin) to the bivalent form (Cob(II)alamin), which can no longer function as a methyl carrier. Their paper, in a journal not perused by many in the biomedical field, remained unnoticed by both haematologists and anaesthetists for 10 years.

In a seminal study, Amess and his colleagues (1978) showed that 24 h of administration of nitrous oxide to patients caused interference with deoxyribonucleic acid (DNA) synthesis, and they correctly inferred that nitrous oxide had interfered with the function of vitamin B<sub>12</sub>. A few months later, Deacon and her colleagues (1978) showed that nitrous oxide did in fact cause rapid inhibition of the activity of methionine synthase in the rat.

Also in the same journal and the same year, Layzer (1978) reported a condition resembling sub-acute combined degeneration of the cord in 15 patients who had been chronically exposed to high concentrations of nitrous oxide. There was thus a parallel with megaloblastic anaemia and a working hypothesis for the agranulocytosis and the teratogenesis which had previously been observed as toxic effects of nitrous oxide.

### *Metabolic changes*

So far as is known, nitrous oxide interacts in the body only with vitamin B<sub>12</sub>. This is the coenzyme for both methionine synthase and methylmalonyl CoA mutase. Vitamin B<sub>12</sub> does not function as a

methyl carrier in the latter enzyme, which is not directly affected by nitrous oxide. Methionine synthase activity is, however, rapidly inhibited in mouse, rat and (more slowly) in man. Recovery is slow and, in the rat, takes several days, probably requiring the synthesis of new apoenzyme and absorption of new unoxidized vitamin B<sub>12</sub>. This is because the oxidation is irreversible and, furthermore, the inactivated B<sub>12</sub> is thought to be irreversibly bound to the apoenzyme.

In the present state of knowledge, it appears that the sole biochemical effect of nitrous oxide is to block the transmethylation reaction shown in figure 1. However, the metabolic consequences are not trivial. There are two products of the reaction, methionine and tetrahydrofolate. Metabolic consequences may be attributable to depletion of either or both products. However, their relative importance is not yet clarified, particularly in the clinical situation where other biochemical abnormalities may be present.

*Methionine and its metabolites* (fig. 2). Methionine is a dietary constituent, but its daily turnover is approximately double the dietary intake. Therefore, methionine is recycled from homocysteine, via S-adenosyl methionine and S-adenosyl homocysteine (fig. 3). Two pathways are available to methylate homocysteine. The first is catalysed by methionine synthase (fig. 1) and is affected by nitrous oxide. The second is the betaine pathway which is shown in figure 3. It is not affected by nitrous oxide, but is induced in the rat liver when the methionine synthase pathway is blocked by nitrous oxide (Lumb et al., 1983).

Catabolism and protein synthesis are normally in balance, but not when patients are catabolic. Conventional parenteral nutrition can easily supply the daily requirement of methionine. In addition to its role in protein synthesis, methionine is important as the precursor of S-adenosyl methionine, which is the direct methyl-group

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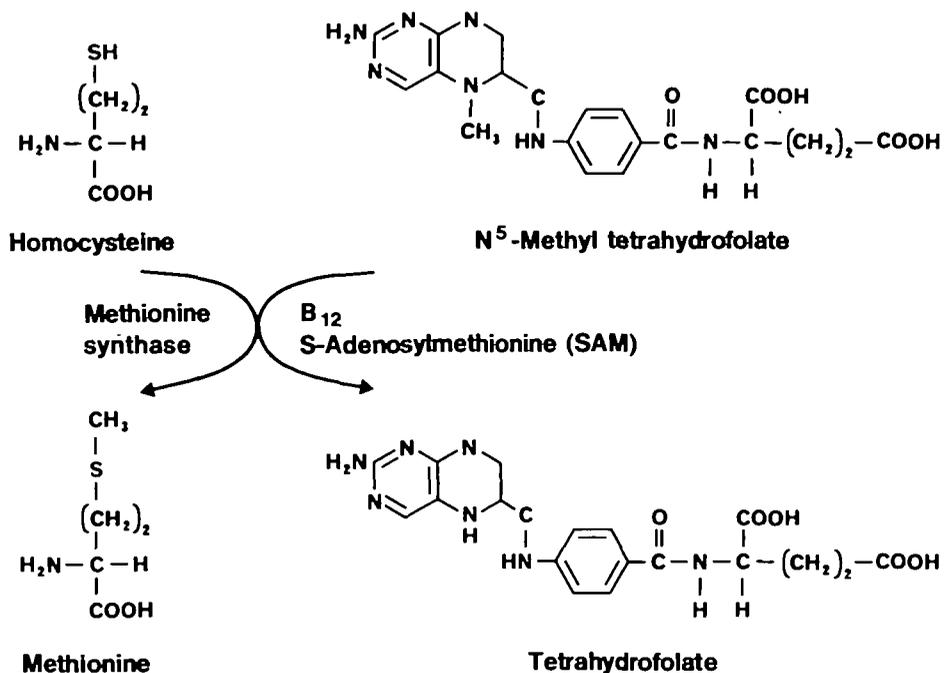


FIG. 1. The transmethylation reaction in which vitamin B<sub>12</sub> is a co-factor for the enzyme methionine synthase.

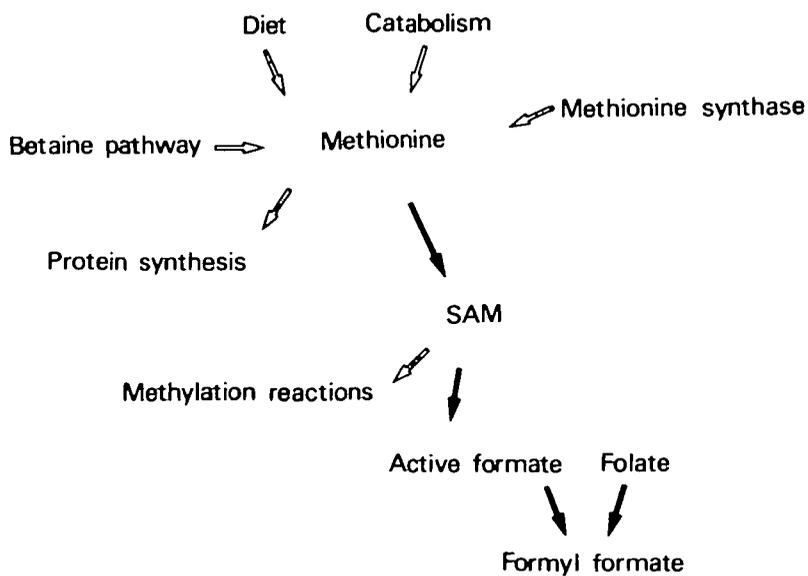


FIG. 2. Factors influencing the balance of methionine and S-adenosyl methionine (SAM).

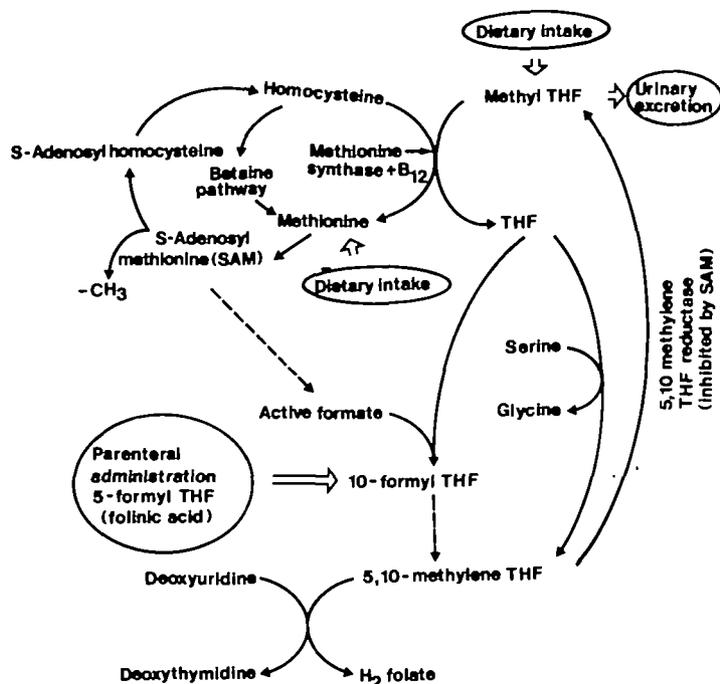


FIG. 3. Simplified metabolic pathway to show the relationship between the methionine synthase reaction and the synthesis of deoxythymidine. THF = tetrahydrofolate.

donor for a large number of methylation reactions including, for example, the conversion of nor-adrenaline to adrenaline, and the synthesis of arachidonic acid. Methylation is also concerned in myelination, and sub-acute combined degeneration of the cord probably results from depletion of S-adenosyl methionine. Of special importance is the production of active formate which reacts with tetrahydrofolate to yield formyl tetrahydrofolate (fig. 3).

In spite of the undoubted effect of nitrous oxide on methionine synthase, there is no measurable change in plasma methionine or S-adenosyl methionine concentrations in man during the first 3 h of anaesthesia with nitrous oxide (Nunn, Sharer et al., 1986), but concentrations of plasma methionine were decreased to 15–25% of the preoperative values between 8 and 24 h of administration of nitrous oxide (Skacel et al., 1983). However, less profound reductions were also observed in two control patients anaesthetized for 24 h with etomidate but no nitrous oxide. These patients reached minimal values of methionine concentration after 12 h of anaesthesia, but the methionine concentration returned to the

preoperative value before the end of the 24 h of etomidate anaesthesia. Nunn, Sharer and colleagues (1986) confirmed earlier observations that routine preoperative starvation reduces the concentration of amino acids in plasma by about 30%. However, S-adenosyl methionine concentrations were unchanged either by preoperative starvation or by 3 h of nitrous oxide anaesthesia.

Two groups have reported on changes in methionine and S-adenosyl methionine concentrations following prolonged exposure of the rat to 50–70% nitrous oxide (Lumb et al., 1983; Viña, Davis and Hawkins, 1986). Results for 24 h exposure are summarized in table I. There is a consistent finding of a reduction in both methionine and S-adenosyl methionine in both studies, although results do not always attain statistical significance. Food was withheld in Viña's study but was available *ad libitum* in Lumb's study. There is, however, good evidence that exposure of the rat to nitrous oxide for shorter durations causes significant reductions in the hepatic concentrations of S-adenosyl methionine. Makar and Tepfly (1983) reported an approximately 50% reduction in the hepatic concentration of

TABLE I. Changes in methionine and S-adenosyl methionine (SAM) concentrations following 24 h exposure of rats to 50–70% nitrous oxide. † Lumb and colleagues (1983): 50% nitrous oxide; numbers too small for statistical analysis. ‡ Viña, Davis and Hawkins (1986): 70% nitrous oxide

	Control	Nitrous oxide	P
<b>Plasma</b>			
Methionine (nmol ml <sup>-1</sup> )			
Lumb†	38	24	
Viña‡	84	69	ns
<b>Liver</b>			
Methionine (nmol g <sup>-1</sup> )			
Lumb†	112	94	
Viña‡	177	98	< 0.05
SAM (nmol g <sup>-1</sup> )			
Lumb†	99	91	
Viña‡	70	53	ns
<b>Brain</b>			
Methionine (nmol g <sup>-1</sup> )			
Lumb†	37	22	
Viña‡	54	29	< 0.05
SAM (nmol g <sup>-1</sup> )			
Lumb†	30.5	28	
Viña‡	23	15	< 0.05

S-adenosyl methionine after 2 h exposure to nitrous oxide and Ells and colleagues (1982) observed a 33% reduction after 4 h.

**Folates** (fig. 3). Deoxythymidine, an essential base in DNA, is synthesized from deoxyuridine and the obligatory carbon donor for this reaction is 5,10-methylene tetrahydrofolate (THF). This is formed by the serine pathway from THF, but in man the more important pathway is from methenyl THF, which is in turn derived from 10-formyl THF. The sequence of events in administration of nitrous oxide in both man and rat is as follows. The first detectable change is interference with the methionine synthase reaction. Shortly afterwards, there is interference with DNA synthesis, manifest by an abnormal deoxyuridine suppression test. This is presumably attributable to depletion of formyl folate resulting from depletion of one or other or both of the products of the transmethylation reactions.

The pattern of depletion of folate compounds during exposure to nitrous oxide is not fully clarified. Total folate concentrations in liver of rats were found to decrease to approximately one-half of control values during 48 h exposure to 50% nitrous oxide (Lumb et al., 1980). During the first 24 h, most of the decrease is attributable to non-methylated folate, indicating trapping in

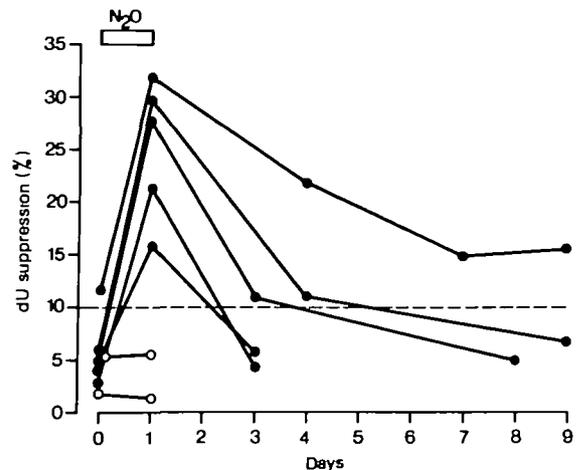


FIG. 4. Time course of changes in deoxyuridine suppression test in patients exposed to nitrous oxide for 24 h (●). Control patients received etomidate instead of nitrous oxide (○). Values over 10% are abnormal in our laboratory. Reproduced with permission from Skacel and colleagues (1983).

the form of methyl THF, as a result of block of the transmethylation reaction. However, there appears to be escape from the folate trap during succeeding days of exposure to nitrous oxide. In contrast to hepatic folate, serum folate increases during exposure to nitrous oxide in man (Skacel et al., 1983). This indicates interference with entry of folate to the cells which requires conversion to folate polyglutamate, the normal intracellular form of all folates.

Interference with DNA synthesis is responsible for the megaloblastic changes and agranulocytosis which follow prolonged administration of nitrous oxide in man, but not in experimental animals. It is probably also the main cause of the fetotoxicity attributable to nitrous oxide.

#### Haematological changes

Megaloblastic changes in bone marrow are consistently found in patients who have been exposed to anaesthetic concentrations of nitrous oxide for 24 h (Amess et al., 1978; Skacel et al., 1983). This is associated with abnormal values for the deoxyuridine suppression which are comparable to those found in pernicious anaemia. It is a consistent finding that exposures to nitrous oxide lasting 4 days or longer result in agranulocytosis (Ablett, 1956; Lassen et al., 1956; Wilson, Martin and Last, 1956). For exposures lasting 24 h, stores of mature polymorphs in the peripheral blood are normally sufficient to prevent a reduction in the

number of granulocytes in the peripheral blood during the interval before recovery of DNA synthesis (fig. 4). However, in two of seven patients who received nitrous oxide for 24–36 h (Skacel et al., 1983) the neutrophil count decreased by the 2nd to 4th days to  $1 \times 10^9$  litre<sup>-1</sup>. There was also an increased proportion of hypersegmented polymorphs in the circulating blood from the 4th to 14th days after operation.

The effect of exposures lasting less than 24 h is not fully clarified and is considered below. There is now considerable evidence that the abnormal deoxyuridine suppression test and the megaloblastic changes can be reversed by treatment with 5-formyl THF (folinic acid) which is converted in the body to 10-formyl THF. This is further considered below.

#### *Teratogenesis*

As expected, nitrous oxide crosses the placenta, and it has been shown in rats that fetal methionine synthase activity is inhibited soon after the effect is demonstrable in the maternal liver (Baden, Serra and Mazze, 1984). Fink, Shepard and Blandau (1967) exposed Sprague–Dawley rats to nitrous oxide for 2 days at the period of maximal organogenesis and observed a highly significant increase in skeletal deformities. Lane and colleagues (1980) extended these observations and showed an increased incidence of resorption and abnormalities (including skeletal) in the same species exposed to 70% nitrous oxide on the 9th day of pregnancy. Xenon had no effect and this convincingly showed it to be specific to nitrous oxide and not a general effect of anaesthesia *per se*. Keeling and colleagues (1986) confirmed Lane's findings and also demonstrated that the effect could, to a large extent, be reversed by pretreatment with folinic acid. This confirmed the view that the fetotoxicity of nitrous oxide was, in fact, the result of depletion of folates, and also suggested a feasible method of avoiding the effect. Pope and colleagues (1978) exposed pregnant rats of the same species to 50% nitrous oxide throughout pregnancy without observing any changes in the offspring other than decreased fetal weight. The betaine pathway of methionine synthesis would have been induced in the first few days of exposure (Lumb et al., 1983).

Extrapolation of data from rats to man is fraught with difficulty. Apart from species differences considered below, the circumstances of exposure of pregnant women to nitrous oxide are different

from the pattern adopted in laboratory exposure of rats. The commonest anaesthetics administered in pregnancy are for relatively short procedures and at the end of the major period of organogenesis. Whatever the explanation, there are now a large number of epidemiological studies which are unanimous in finding no increased incidence of fetal malformations following the administration of anaesthetics in pregnancy (Smith, 1963; Snider and Webster, 1965; Brodsky et al., 1980; Duncan et al., 1986; Konieczko, Chapple and Nunn, 1987).

#### *Time course of inhibition of methionine synthase and DNA synthesis*

*Methionine synthase activity.* Investigation of the major biochemical effects of nitrous oxide require sampling of tissue (liver, bone marrow and brain) and it is understandable that most of the work has been undertaken in experimental animals, particularly rodents. Nevertheless, it is becoming apparent that there are important species differences between rat and man in the time course of inhibition.

In rodents, inhibition of methionine synthase is more than 50% in less than 30 min of exposure to nitrous oxide (Deacon et al., 1978; Koblin et al., 1981). Studies in progress by Minty with the author (unpublished) have shown that, in fact, methionine synthase activity is reduced to less than 50% after only 5 min exposure of Sprague–Dawley rats to 50% nitrous oxide.

The time course is very different in man. In a series of seven liver biopsies reported by Koblin and colleagues (1982), inhibition averaged 50% after about 2 h exposure to a mean concentration of 60% nitrous oxide. An even slower onset of inhibition of methionine synthase activity of bone marrow in patients anaesthetized with nitrous oxide was reported by Kano and colleagues (1981). Current studies by the author and Minty (unpublished) fully confirm that inhibition is slower in man than in rodents by an order of magnitude, although there is considerable individual variation in individual residual methionine synthase activity after various periods of exposure. They have found some patients with negligible activity after 30 min, but other patients have some residual activity up to 2 h exposure. Landon and Toothill (1986) found methionine synthase activity in human placenta to be normal following delivery after Caesarean section with nitrous oxide

exposure of less than 25 min up to the time of delivery of the placenta.

For exposures lasting 4 h, the dose-response curve in the rat (Koblin et al., 1981) indicates an  $ED_{50}$  of about 10% nitrous oxide. Concentrations in use in clinical anaesthesia (usually 50–70%) are therefore on the upper flat part of the dose-response curve and there should be no major differences attributable to the concentration of nitrous oxide selected within this range. The dose-response curve has not been determined for man.

**Plasma methionine concentration.** This has been considered above. There are no changes during the first 3 h of anaesthesia with nitrous oxide in man (Nunn, Sharer et al., 1986), but values were 30% of those before operation after 8 h and 20% after 24 h (Skacel et al., 1983). S-Adenosyl methionine was unchanged after 3 h.

**Deoxythymidine synthesis.** There is agreement that there are grossly abnormal values for the dU suppression test with megaloblastic marrow in man after 24 h exposure to nitrous oxide (Amess et al., 1978; O'Sullivan et al., 1981; Skacel et al., 1983). O'Sullivan and colleagues (1981) reported abnormal marrow in four of five patients exposed to nitrous oxide for 12 h.

There is a difference in the findings reported after shorter exposures. O'Sullivan found no abnormalities of marrow in 30 routine surgical patients who had received nitrous oxide for less than 6 h. However, Amos and colleagues (1982) reported abnormal dU suppression tests and megaloblastic marrow in a large series of patients who received nitrous oxide for 6 h or less (fig. 5). Their patients all required intensive therapy and raise the possibility that the compromised patient may be more sensitive to nitrous oxide. They reported that 18 of the 22 megaloblastic patients died. A patient with a severe haemorrhage was found to have an abnormal dU suppression and megaloblastic marrow after exposure to nitrous oxide for only 2 h (Nunn, Chanarin et al., 1986). Kano and colleagues (1981) found abnormal dU suppression tests in some patients after 4 h exposure to nitrous oxide and no normal values in exposures of more than 7 h.

We must conclude from these data that interference with thymidine synthesis is to be expected in man after 12 h of exposure to nitrous oxide, but may appear within 2 h or even less.

Number of patients	13	20	9
Mean $\pm$ SEM	7.9 $\pm$ 0.8	11.9 $\pm$ 1.1	21.3 $\pm$ 2.9

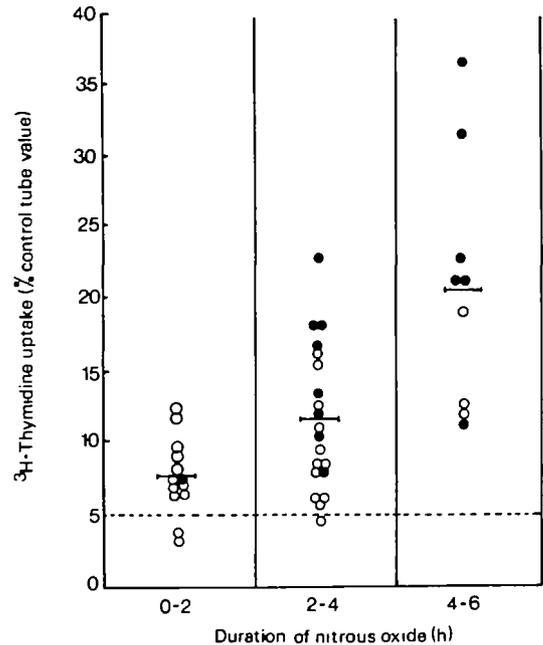


FIG. 5. Postoperative deoxyuridine suppression tests (expressed as  $^3\text{H}$ -thymidine uptake) in patients who had received nitrous oxide for different durations. ● = Megaloblastic marrow. Values over 5% are abnormal from these authors' laboratory. Reproduced with permission from Amos and colleagues (1982).

There is considerable individual variation and the sick patient may be more susceptible.

#### Biochemical correction of the effects of nitrous oxide

**Methionine.** It is possible to supply methionine by the oral route to cover any shortfall resulting from loss of activity of methionine synthase. This was investigated in relation to a neurological condition resembling subacute combined degeneration of the cord, induced in monkeys by chronic exposure to 15% nitrous oxide (Scott et al., 1981). Supplementation of diet with methionine 2 g daily greatly reduced the demyelination and neurological damage which was seen in the monkeys which did not receive the dietary supplementation.

**Folic acid.** The administration of folic acid (5-formyl THF) cannot restore the concentrations of either of the products of the methionine

synthase reaction, but it can restore deoxythymidine synthesis. The required dose in man is large. However, there is now very strong evidence that 30 mg twice daily will prevent development of an abnormal dU suppression test and megaloblastic marrow changes in some, but not all, patients during prolonged exposure to nitrous oxide (O'Sullivan et al., 1982; Skacel et al., 1982 (supplement to letter); Amos et al., 1984; Kano et al., 1984; Nunn, Chanarin et al., 1986). Reference has been made above to the mitigation of the fetotoxic effects of nitrous oxide in rats by pre-treatment with folinic acid (Keeling et al., 1986). Kano and colleagues have also reported that methylcobalamin at the end of operation corrected the abnormal dU suppression tests. This unexpected finding suggests that the bond between the methionine synthase apoenzyme and oxidized vitamin B<sub>12</sub> may be broken. It is to be expected that any B<sub>12</sub> preparation administered *during* exposure to nitrous oxide would be rapidly oxidized to bivalent cobalt.

#### CONDITIONS REQUIRING SPECIAL CONSIDERATION WHEN NITROUS OXIDE IS USED

The final section of this paper considers specific situations in which nitrous oxide may be administered. In some cases nitrous oxide appears to be contraindicated, while in others it appears to be harmless. However, there are many situations in which it is not yet possible to form a clear opinion. It must be stressed that this review only considers the interaction between nitrous oxide and vitamin B<sub>12</sub>. It does not consider other properties of nitrous oxide which might influence its choice as the best anaesthetic agent in a particular situation.

##### *Administration lasting more than 24 h*

Gross interference with DNA synthesis is to be expected after 24 h of administration (Amess et al., 1978; Skacel et al., 1983) and production of granulocytes will be seriously impaired. In patients with normal bone marrow, stores of mature granulocytes will normally be adequate to prevent leucopaenia for up to about 3 days. Beyond that time, however, leucopaenia will usually develop and agranulocytosis has been reported after 5–7 days (Lassen et al., 1956; Wilson, Martin and Last, 1956).

There would seem to be an absolute contraindication to the continued use of nitrous oxide after 24 h, although the precise upper limit of

safe duration cannot be deduced from existing data and is probably variable from one patient to another, depending *inter alia* on marrow stores of granulocytes. If there are very strong indications for the continued use of nitrous oxide, there is good evidence (outlined above) that protection from megaloblastic changes can be obtained, in most but not all patients, with folinic acid 30 mg twice daily. Since efficacy does not seem to be 100%, it is important to monitor with daily full blood counts.

##### *24-Administration*

A reasonably healthy surgical patient appears able to receive nitrous oxide for 24 h without overt harm, although there will almost certainly be megaloblastic marrow changes and an abnormal dU suppression test. None of the patients reported by Amess and colleagues (1978) became leucopaenic in the postoperative period. However, in two patients reported by Skacel and colleagues (1983), the neutrophil count decreased to  $1 \times 10^9$  litre<sup>-1</sup> between the 2nd and 4th days. These changes can usually be prevented by the use of folinic acid. The excellence of the analgesia provided by nitrous oxide may well be considered to outweigh the haematological problem in the healthy surgical patient.

##### *Administrations lasting less than 24 h*

It seems likely that in man, in contrast to the rat, exposure of less than 30 min will not cause any measurable change in hepatic methionine synthase activity. In combination with a wealth of clinical experience, this suggests that there is no special hazard for short exposures to nitrous oxide. There is a variable response to exposures lasting between 30 min and 2 h. However, it now seems likely that exposures of more than 2 h are likely to cause interference with hepatic methionine synthase activity. The paucity of human data makes it more difficult to say how long an exposure is required to cause significant interference with DNA synthesis. It is likely that there will be considerable individual variation and results obtained in healthy patients cannot be extrapolated to the patient who is seriously ill. Nevertheless, it seems likely that, once methionine synthase activity is inhibited, it will remain so for some days. It is therefore possible that an abnormal dU suppression test may develop after the end of surgery and exposure to nitrous oxide. This has not been investigated in man.

*Repeat exposures to nitrous oxide*

Since the inhibition of methionine synthase is rapid and its recovery slow (Deacon et al., 1980), it is to be expected that exposure repeated at intervals of less than 3 days will have a cumulative effect. A patient exposed to Entonox (50% nitrous oxide + 50% oxygen) for 15 min three times a day twice developed an abnormal dU suppression test and megaloblastic marrow changes after total exposures of about 2 weeks (Nunn et al., 1982).

Megaloblastic marrow and an abnormal dU suppression test were found at the start of an anaesthetic given 7 h after a previous anaesthetic with nitrous oxide lasting 2 h (Nunn, Chanarin et al., 1986). Nitrous oxide was given a second time, but with 30 mg of folic acid, after which the marrow was found to be normal.

There appear to be no other biochemical studies after repeat anaesthetics in man. However, clinical experience suggests that the outcome is often more favourable than might be expected, particularly in children who often require repeated anaesthetics. R. M. Jones (personal communication) has kindly supplied data on three children who have recently had 25–40 daily administrations of nitrous oxide for radiotherapy, each lasting about 20 min. Leucocyte counts in peripheral blood have remained unchanged.

*The sick patient*

Reported results for the dU suppression test tend to show abnormalities after shorter exposures to nitrous oxide in "sick" patients, compared with relatively healthy surgical patients. The patients reported by Amos and colleagues (1982) were all sufficiently "sick" to be admitted to Intensive Care and included 50 patients who had received nitrous oxide for durations up to 6 h. Of these patients, 48 had abnormal dU suppression tests and 18 had megaloblastic marrow—even one who had received nitrous oxide for less than 2 h. This was also seen in the case report described above (Nunn, Chanarin et al., 1986). These findings contrast with those of O'Sullivan and colleagues (1982) who, in relatively healthy patients, observed no abnormal marrows when the duration of exposure to nitrous oxide was 6 h.

It thus seems quite likely that the safe period of exposure to nitrous oxide is influenced by the preoperative state of the patient, but it is difficult to be more precise. There is, again, a paucity of human data and great difficulty in defining what we mean by "sick".

There may be considerable clinical relevance in megaloblastic changes occurring in the postoperative period in the sick patient. In Amos' series, 16 of 18 patients with megaloblastic changes died while in the Intensive Care Unit. This may be compared with a mortality of only seven of 22 patients who remained normoblastic in spite of having received nitrous oxide. It is generally recognized that mortality during intensive care is related to the number of systems in failure and marrow failure may be sufficient to tip the balance in a patient who already has multi-system failure.

There are no data on the additional risk imposed by using nitrous oxide for anaesthesia in a patient who is gravely ill. A typical example would be the closure of a stress ulcer in a patient with multi-system failure. This is clearly a very difficult field for a controlled randomized prospective trial, but that is the only way that answers, in terms of clinical relevance, will emerge.

*Wound healing and infection*

It would seem that interference with DNA synthesis might be unfavourable for wound healing. An animal study by Algie and colleagues (1984) failed to show any adverse effect of nitrous oxide. It would be valuable to determine whether there is any effect of nitrous oxide on the ability of a patient to survive a severe infection, but a trial to test this hypothesis would be a formidable undertaking and no reports are yet forthcoming.

*Pregnancy*

The demonstration of no change in placental methionine synthase activity following the use of nitrous oxide at Caesarean section suggests that it is unlikely that the fetus would be affected by the short duration of exposure (Landon and Toothill, 1986). The definitive experiment would be a study of the fetal liver following delivery, but ethical considerations would seem to exclude this investigation. Changes in the rat fetal liver (Baden, Serra and Mazze, 1984) are probably not relevant because of the very different rate of inactivation of human and rodent methionine synthase.

The use of nitrous oxide during early pregnancy presents a very difficult problem. On the one hand, there is irrefutable evidence that nitrous oxide is a mild teratogen in the rat and its partial reversal with folic acid suggests very strongly that the mechanism is interference with deoxythymidine synthesis, secondary to inhibition of fetal methionine synthase activity (Keeling et al., 1986). On

the other hand, a very large body of human epidemiological evidence (cited above) has failed to find any increase in the incidence of fetal abnormalities following anaesthesia in pregnancy. There are three possible explanations. First, there may be a species difference and this cannot be directly tested. Second, most anaesthetics given in pregnancy are of short duration and may be too short to affect fetal methionine synthase activity. Third, the commonest procedure requiring an anaesthetic in pregnancy is cervical suturing and this is usually undertaken after most major organogenesis is completed.

The potentially dangerous situation would be an anaesthetic lasting several hours and administered within the first 4 weeks of pregnancy. There is one report of a normal fetus delivered after the mother received nitrous oxide for 2 h approximately 2 weeks after conception (Park, Fulton and Shelley, 1986). Clearly, this is an uncommon event and it will take a long time to assemble a sufficient number of such cases to permit a conclusion to be drawn on the risk. In the meantime, it is the view of the author that the use of nitrous oxide in pregnancy during the period of organogenesis is inadvisable in the light of present knowledge. There are a large number of alternative anaesthetic agents for which there is no convincing evidence of teratogenic potential.

An alternative approach would be to cover the administration of nitrous oxide during early pregnancy with folic acid, following the animal studies reported by Keeling and colleagues (1986). This has been considered by Marx (1985), and there is no obvious objection to the practice.

#### *Exposure to trace concentrations*

The dose-response curve for nitrous oxide on methionine synthase activity is displaced to the left by increasing durations of exposure (Sharer et al., 1984). In the rat, maximal sensitivity occurred after 48 h exposure and the ED<sub>50</sub> for inhibition of hepatic methionine synthase was then about 5000 parts per million (p.p.m.). The lowest concentration at which partial inhibition could be detected was 1000 p.p.m. and no changes were detected at 450 p.p.m. These values are generally above concentrations recorded in operating theatres, even without scavenging equipment, and they are far above the low concentrations which can be attained with scavenging (Davenport et al., 1980). Normal serum methionine concentrations have been reported in staff working in badly contami-

nated operating theatres (Nunn et al., 1982). It seems unlikely that normal work in operating theatres exposes staff to any danger arising from inactivation of vitamin B<sub>12</sub>. It should, however, be stressed that the dose-response curve for chronic exposure was prepared for rats and it is difficult to see how it could be determined in man.

Dental operations are a different matter and much higher levels of contamination have been reported, sometimes in excess of 5000 p.p.m. (Hillman et al., 1981). The difference is attributable to high flow rates of nitrous oxide and poor ventilation. Sweeney and colleagues (1985) reported three instances of abnormal dU suppression test in the bone marrow of 20 dentists using nitrous oxide for analgesia. Two dentists had hypersegmented polymorphs in their peripheral blood.

#### *Abuse of nitrous oxide*

The report of Layzer (1978) makes chilling reading. Fifteen patients had been inhaling subanaesthetic concentrations of nitrous oxide for long periods of time, 14 of them intentionally. All had a condition resembling subacute combined degeneration of the cord, which in most cases responded to abstinence from nitrous oxide. Other unpublished examples have been reported to the author and there seems no doubt that this is an established consequence of the abuse of nitrous oxide. Scott and colleagues (1981) reported a similar condition in monkeys which responded to supplementation of dietary methionine. The parallel with deficiency of vitamin B<sub>12</sub> would suggest that folic acid would be ineffective. The possibility of abuse of nitrous oxide should be considered in any patient with access to the gas who reports signs and symptoms resembling subacute combined degeneration of the cord.

#### *The patient with subclinical B<sub>12</sub> deficiency*

It would not be surprising if a patient with subclinical vitamin B<sub>12</sub> deficiency should develop symptoms following administration of nitrous oxide, which presumably inactivates stores of vitamin B<sub>12</sub> as well as B<sub>12</sub> bound to methionine synthase. Schilling (1986) has recently reported two women who developed neurological dysfunction consistent with subacute combined degeneration of the cord within 8 weeks of being anaesthetized with nitrous oxide. One had a previous ileal resection for Crohn's disease and the other had pernicious anaemia. O'Leary,

Combs and Schilling (1984) have demonstrated, in animals, synergism between vitamin B<sub>12</sub> deficiency and exposure to nitrous oxide.

The only source of vitamin B<sub>12</sub> in man is dietary intake from animal products. Strict vegetarians may have a subclinical deficiency and might prove to be at risk from nitrous oxide administration.

#### *Methotrexate therapy*

It has recently been suggested that nitrous oxide may complicate methotrexate therapy in children. Ueland and colleagues (1986) concluded that it might reduce the therapeutic effect and increase side effects. However, this theoretical problem has not yet been investigated.

#### CONCLUSIONS

If nitrous oxide were to be introduced as a new drug, the metabolic effects outlined above would raise grave doubts about its acceptability. Eger (1985) has presented the case against nitrous oxide. He included a range of adverse effects, but the most serious are based on the interaction with vitamin B<sub>12</sub>. Eger himself (personal communication) has now abandoned the use of nitrous oxide in routine clinical anaesthesia. The alternative case in favour of the continued use of nitrous oxide has been presented by Saidman and Hamilton (1985). They make the telling point that, except in the case of prolonged administration, inhibition of methionine synthase has not produced a clinical effect recognized by generations of skilful observers.

The contradiction between the serious biochemical effects of nitrous oxide and the apparent absence of adverse clinical effects in routine anaesthesia makes it difficult to draw firm conclusions. On the one hand, some may feel that the biochemical effects require that nitrous oxide should be abandoned in favour of alternative anaesthetics, of which there are plenty. On the other hand, the vast majority of anaesthetists, including the author, are influenced by the paucity of information on adverse outcome and continue to use such a well tried agent. The truth probably lies between these two extreme viewpoints. Evidence has been presented for believing that nitrous oxide may be quite harmless when used for a limited duration in patients without adverse predisposing factors. The definition of a safe duration is a difficult problem and is considered above. What are the adverse predisposing con-

ditions is an even more difficult question. The more obvious factors have been considered, but a great deal of difficult and detailed clinical study will be required to define those conditions in which nitrous oxide may be contraindicated.

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