The effect of sepsis and short-term exposure to nitrous oxide on the bone marrow and the metabolism of vitamin B_{12} and folate

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Summary

It is recognised that prolonged anaesthesia with nitrous oxide (N_2O) induces megaloblastic anaemia by oxidising vitamin B_{12}. To determine whether sepsis aggravates the effect of N_2O on haemopoiesis 5 patients with severe sepsis, who required surgery and were exposed to short-term (45 - 105 minutes) N_2O anaesthesia, were studied. None had evidence of pre-operative vitamin B_{12} or folate deficiency. The effect of the combination of N_2O anaesthesia and sepsis on DNA synthesis in bone marrow cells was assessed morphologically, and by the deoxyuridine suppression test. In 3 patients exposed to the longest duration (75 - 105 minutes) of N_2O, addition of folinic acid and vitamin B_{12} partially improved the utilisation of deoxyuridine *in vitro*. No patient had evidence of megaloblastic haemopoiesis as judged by bone marrow morphology. It is concluded that prolonged N_2O anaesthesia in patients with severe sepsis may adversely affect DNA synthesis. Although this effect did not manifest as overt megaloblastic erythropoiesis, it may be prudent to avoid N_2O in such patients.

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The effect of prolonged exposure to nitrous oxide (N_2O) on haemopoiesis is well recognised.[1] Leucopenia and thrombocytopenia were described by Lassen *et al.*[2] in 1956 in patients treated for tetanus with N_2O for periods of 4 - 17 days. Megaloblastic changes were noted in the bone marrow of some of the patients. Amess *at al.*,[3] utilising the deoxyuridine suppression test (dUST) with or without added vitamin B_{12}, suggested that prolonged N_2O administration interferes with the function of vitamin B_{12} by oxidising the cobalt of the vitamin.

The induction of megaloblastosis by N_2O may, however, be much more rapid in complicated situations. In a study of 50 patients admitted to an intensive care unit after major surgery, abnormal deoxyuridine utilisation for DNA synthesis was observed in 11 of 13 patients who received N_2O for 2 hours or less;[4] the dUST proved to be more sensitive than bone marrow morphology in demonstrating the defect in vitamin B_{12} metabolism.

Gross sepsis is still common in overpopulated areas where the standards of hygiene are not optimal. Also, the decision to consult a doctor is often delayed — with disastrous results for the patient. The prognosis of such patients may be improved if all factors that may affect the course of the disease are taken into account. In this regard, it is not known whether the combination of N_2O anaesthesia and serious sepsis may precipitate acute megaloblastic haemopoiesis.

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A study was undertaken to test the hypothesis that sepsis may contribute to the effect of N_2O on vitamin B_{12} and folate metabolism thus exacerbating the degree of megaloblastic haemopoiesis in those patients with sepsis requiring surgery.

Patients and methods

Five patients with sepsis and requiring surgery were studied in a project approved by the Ethical Committee of the University of the Orange Free State. Patient details are given in Table I. Four patients had abdominal surgery. The exposure to N_2O varied from 45 minutes to 105 minutes.

The severity of sepsis was graded according to a modification of the classification of Skau *et al.*[5] and Lebute and Stoner.[6] The simple numerical score was based on: type of sepsis, pyrexia, secondary effects of sepsis, and relevant laboratory data (Table II). Only patients with a score of 7 or more were admitted to the study.

Patients were excluded from the study if they: (*i*) suffered from any haematological disease except anaemia due to haemorrhage; (*ii*) were treated with drugs known to affect vitamin B_{12} or folate metabolism (e.g. anti-epileptic drugs, immunosuppressive medication or chemotherapy); or (*iii*) had clinical or laboratory evidence of chronic liver disease.

Protocol and anaesthesia

The patient's general condition was noted and in preparation for emergency surgery, 10 mg metoclopramide was given intravenously and 15 ml of magnesium trisilicate administered orally. Pre-operative and laboratory tests included measurement of blood gases; a full blood count; assay of red cell and serum folate, and serum vitamin B_{12} levels (SimulTRAC-SNB, Becton Dickinson, Orangeburg, NY, USA); a biochemical profile (SMAC, Technicon Instruments); and a routine coagulation screening consisting of measurement of prothrombin and activated partial thromboplastin time.

After pre-oxygenation, sleep was induced with thiopentone 3 mg/kg, cricoid pressure applied, and intubation facilitated with suxamethonium 1,5 mg/kg. Anaesthesia was maintained with low doses of halothane and/or fentanyl. After the aspiration of a bone marrow sample, 50 - 70% N_2O was included in the anaesthetic.

After surgery, the patients were admitted to an intensive care unit. A bone marrow aspiration was repeated 24 hours later and serum and red cell folate and serum vitamin B_{12} levels again estimated. The full blood count and biochemical profile were repeated daily for 7 - 8 days postoperatively.

The dUST was performed on both intra- and postoperative bone marrow aspirates, as described.[7]

Results

Pre-operatively, 3 patients had a moderate normochromic normocytic anaemia. Patient 2 had a pancytopenia and patients 2 and 4 had a generalised bleeding tendency manifesting as

TABLE I. PATIENT DETAILS

Patient	Sex, age (yrs)	Diagnosis	Sepsis score	Operation	Duration of exposure to N_2O (min)
1	M, 19	Perforated bowel	7	Laparotomy	75
2	F, 23	Puerperal sepsis	14	Hysterectomy; bilateral salpingo-oophorectomy	75; 45 (2nd laparotomy)
3	M, 18	Ruptured appendix + abscess	10	Laparotomy; drainage of abscess	90
4	F, 28	Puerperal sepsis	10	Hysterectomy; bilateral salpingo-oophorectomy	105
5	M, 23	Bilateral empyema and mediastinitis	10	Drainage of empyema	45

TABLE II. SEPSIS SCORE

2 points

- Chill or fever > 38,9°C or hypothermia < 35,6°C
- Tachypnoea > 28/min or partial arterial carbon dioxide pressure ($Paco_2$) < 32 mmHg
- Pao_2 < 60 mmHg (not due to pre-existing lung disease)
- Hypotension < 90 mmHg or tachycardia > 110/min
- Generalised peritonitis or deep-seated infection, e.g. pelvic abscess
- Jaundice (not due to pre-existing liver disease)

1 point

- Metabolic acidosis
- Elevated liver enzyme values
- Oliguria or elevated serum urea or creatinine values
- Thrombocytopenia or evidence of disseminated intravascular coagulation
- Positive blood culture

bleeding at sites of surgical incision and venepuncture. This was ascribed to thrombocytopenia on day 1 and day 2 post-operatively (Table III).

Two patients had a neutrophil leucocytosis and 1 was neutropenic. There was no hypersegmentation of the neutrophil nucleus or shift to the left observed in any of the patients.

All patients had normal vitamin B_{12} and folate status. This was reflected by normal serum vitamin B_{12} and red cell folate levels. In patient 3 red cell folate could not be assayed because the blood specimen was lost; however, his serum folate was normal (Table IV).

The dUST was performed on bone marrow samples collected pre-operatively and 24 hours postoperatively. Patient 2 had a second exposure to N_2O and the dUST was repeated 24 hours later.

In patients 3 and 4, after exposure to N_2O, more than 10% of DNA synthesis could be ascribed to ^{3}H-thymidine after pre-incubation of the marrow with deoxyuridine. This relative lack of suppression was restored to normal by addition of either vitamin B_{12} or and folinic acid. Methyltetrahydrofolate also corrected the defect in patient 3. Patient 3 had an abnormal dUST before anaesthesia. In all other instances the dUST was within normal expected limits, i.e. less than 10% of the ^{3}H-thymidine was used for DNA synthesis when the bone marrow had been incubated with deoxyuridine.

Bone marrow

In all patients the pre-operative and post-anaesthesia bone marrow was of normal cellularity and haemopoiesis was normal. In particular, there was no evidence of megaloblastic erythropoiesis and giant metamyelocytes and staff cells were absent.

TABLE III. PRE-OPERATIVE LABORATORY DATA

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Reference values
Haemoglobin (g/dl)	13,90	10,10	13,00	8,70	11,20	12 - 16,5
Leucocyte count ($\times 10^9$/dl)	12,39	6,51	22,52	20,99	12,46	4 - 11,0
Platelet count ($\times 10^9$/dl)	357,00	31,00	433,00	114,00	196,00	150 - 400
MCV (fl)	87,20	86,00	91,00	87,00	95,00	80 - 99
Calcium (mmol/l)	2,28	1,82	2,50	1,93	1,89	2,2 - 2,6
Urea (mmol/l)	4,40	4,50	6,40	57,60	11,40	2,6 - 6,7
Creatinine (mmol/l)	8,60	8,70	8,30	8,86	7,80	0,12 - 0,55
LDH (U/l)	295,00	878,00	233,00	785,00	475,00	100 - 350
Albumin (g/l)	29,00	22,00	34,00	26,00	23,00	38 - 52
Cholesterol (mmol/l)	2,38	1,61	1,36	2,91	1,24	2,10 - 5,80

TABLE IV. VITAMIN B_{12} AND FOLATE STATUS AND METABOLISM

Patient	Vit B_{12} (ng/l)	Folate (μg/l)	Red cell folate (μg/l)	Code	dUST (% of control tube) Du only	Du + Vit B_{12}	Du + folinic acid	Du + methyl THF
1	765	3	423	B	7,6	5,0*	6,0	7,6
				A	5,0	4,6	5,8	5,0
2	1 307	2	263	B	3,7	4,0	4,0	3,8
				A	7,1	4,2	4,7	9,5
				A_2	6,6	5,4	3,7	9,2
3	251	7	ND	B	12,3	9,8	2,5	10,2
				A	10,6	8,2	2,6	11,0
4	> 2 000	5	569	B	7,7	5,2	7,0	7,2
				A	12,2	9,1	6,8	8,1
5	> 2 000	7	493	B	6,8	6,3	4,9	ND
				A	9,0	4,7	8,1	ND

Code: B = before exposure to N_2O; A = 24 h after exposure to N_2O; A_2 = 24 h after second laparotomy.
Normal serum values: Vitamin B_{12} = 200 - 900 ng/l; folate = 3 - 20 μg/l; red cell folate = 160 - 640 μg/l.
Du = deoxyuridine.

Biochemical profile

The serum albumin and cholesterol levels were reduced in all patients. This can probably be ascribed to malnutrition. Patients 2 and 4 showed increased levels of serum calcium, urea, creatinine and lactate dehydrogenase (LDH). Serum LDH levels were not elevated to the extent seen in megaloblastic anaemia (Table III).

Discussion

Amess *et al.*[3] studied the effect of N_2O in patients who had cardiac bypass surgery. They demonstrated that prolonged exposure (24 hours) to the gas induced megaloblastic haemopoiesis. These authors, utilising the dUST, correctly inferred that the N_2O affected the metabolism of vitamin B_{12}. N_2O oxidises vitamin B_{12} *in vitro* from the cob(I)alamin to the inactive cob(III)alamin form thus blocking the availability of tetrahydrofolate required for the conversion of deoxyuridine to thymidine (Fig. 1).

Vitamin B_{12} is the co-enzyme for methionine synthase and Deacon *et al.*[8,9] proved that N_2O rapidly inhibits the activity of the enzyme in the rat. Such inhibition, in both man and rat, soon interferes with DNA synthesis. This can be demonstrated by an abnormal dUST. Thus the dUST becomes abnormal in patients exposed to N_2O before morphological changes become evident in the bone marrow.

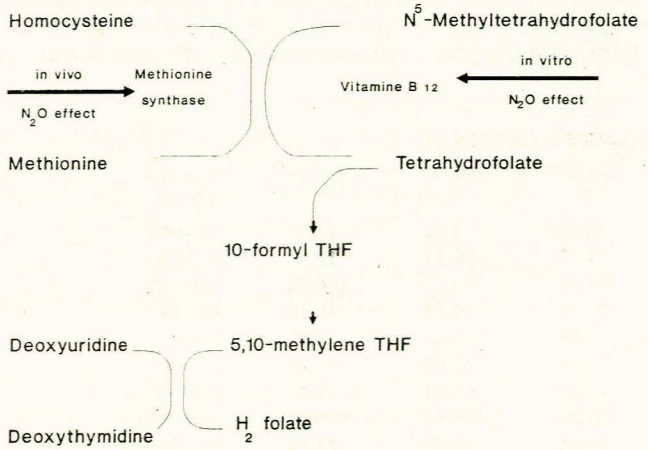

Fig. 1. N_2O oxidises vitamin B_{12} *in vitro*, and *in vivo* inhibits the activity of methionine synthase. These effects interfere with DNA synthesis. This may result in megaloblastic haemopoiesis.

N_2O anaesthesia was the cause of megaloblastic anaemia in severely ill patients admitted to an intensive care unit.[4] In this study 18 of 22 patients had been exposed to N_2O for only 2 - 6 hours. In these surgical patients with a variety of diseases (but no sepsis), there was a clear relationship between the degree of abnormalities of the dUST and the duration of N_2O anaesthesia. Noteworthy was the finding that the dUST was more abnormal in these critically ill patients and its return to normal was slower compared with patients subjected to cardiac surgery. The mortality rate was also strikingly higher in those patients with megaloblastic bone marrow changes. In another study, a patient with severe haemorrhage had an abnormal dUST and megaloblastic marrow after exposure to N_2O for only 1 hour.[10]

The anaemia of chronic disorders and inflammation is complex and the aetiology is multifactorial. Although the most important cause is considered to be a defect in haem synthesis,[11] the anaemia may also be associated with depression of bone marrow function and a maturation arrest of the marrow precursors.[12] Megaloblastic anaemia may also play a role in the development of anaemia in such patients, especially if they are very ill. Thus severe infection[13,14] or fever[15] may accelerate the development of folate deficiency in the critically ill. The role of vitamin B_{12} deficiency in these instances is not known. Shnier and Metz[14] noted that some infants with laboratory evidence of folate deficiency and concomitant malnutrition and infection respond to therapy with either oral folic acid or intramuscular vitamin B_{12}. These authors explained this finding by suggesting that infection *per se* may precipitate megaloblastic marrow changes.

Other factors may also contribute to the rapid development of megaloblastic anaemia in severely ill patients. Examples of these are intravenous feeding with amino acid-ethanol solutions[16] and mild pre-existing vitamin B_{12} deficiency.[17]

It is evident from the foregoing that patients with severe infections exposed to even relatively short periods of N_2O anaesthesia may, theoretically, be at risk. In patients 1, 2 and 5 there were no abnormalities of the dUST. The dUST of patient 3 was abnormal before and after exposure to N_2O. These defects could be corrected by the addition of folinic acid to the deoxyuridine incubation medium. Patient 4 also had an abnormal dUST after exposure to N_2O; this was corrected by folinic acid.

There were no morphological abnormalities suggestive of megaloblastic haemopoiesis in the bone marrow aspirates or peripheral blood cells of the patients. Assay of appropriate serum and red cell samples indicated that none of the patients had a pre-existing folate or vitamin B_{12} deficiency.

The results of the dUST deserve comment. In patients 2, 3 and 4 the postoperative dUST was corrected by either folinic acid or B_{12}. Methyltetrahydrofolate was relatively ineffective and corrected the dUST only of patient 4. This finding suggests that there was indeed an abnormality of vitamin B_{12} metabolism, and not of folate in these patients. These three patients had the longest exposure to N_2O; N_2O exposure for 75 - 105 minutes is within the intermediate range and may or may not affect methionine synthase activity.

We therefore conclude that relatively short-term N_2O exposure may have an adverse effect on vitamin B_{12} metabolism in patients with severe sepsis and no pre-existing vitamin B_{12} or folate deficiency. Alternatively, such co-existent infection may accentuate the well-known effects of N_2O on vitamin B_{12} metabolism. This effect on DNA synthesis can only be demonstrated with the dUST.

Although we could not demonstrate that brief exposure to N_2O in patients with severe sepsis induces overt megaloblastic haemopoiesis, it would nevertheless seem prudent to avoid this anaesthetic agent in such severely ill patients. This is particularly so because in severe sepsis, methionine and tetrahydrofolate metabolism may be affected by other unknown factors. These factors may further add to the adverse effects of N_2O anaesthesia in such patients.

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