

# Toxicity of bone marrow in dentists exposed to nitrous oxide

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## Abstract

The morphology of the bone marrow of 21 dentists who habitually used nitrous oxide in their surgeries was investigated. Exposure to nitrous oxide was measured with an atmospheric sampling device, and each dentist was invited to fill in a questionnaire giving details of medical history, diet, and intake of alcohol. During the trial a full neurological and haematological investigation was carried out and a bone marrow aspirate was examined both morphologically and by the deoxyuridine suppression test. Mean exposures to nitrous oxide ranged from 159 to 4600 parts per million. In all subjects serum vitamin B<sub>12</sub> and folate concentrations were within normal limits. Abnormal results of deoxyuridine suppression tests were obtained in three of the 20 dentists tested; two of these three had abnormal white cells in their peripheral blood films.

This study provides direct evidence that occupational exposure to nitrous oxide may cause depression of vitamin B<sub>12</sub> activity resulting in measurable changes in bone marrow secondary to impaired synthesis of deoxyribonucleic acid.

## Introduction

Inhalation of nitrous oxide inactivates vitamin B<sub>12</sub> by oxidation, which may lead to impaired synthesis of deoxyribonucleic acid (DNA) in the bone marrow with the development of megaloblastic haemopoiesis.<sup>1-3</sup> Several studies have shown that such changes can occur in patients exposed to nitrous oxide over prolonged periods,<sup>4</sup> after multiple short term exposures,<sup>5</sup> and in the period immediately after operation.<sup>6</sup> The possibility that nitrous oxide could inhibit vitamin B<sub>12</sub> activity in much lower concentrations was investigated by Sharer *et al*, who detected significantly decreased activity of the vitamin B<sub>12</sub> dependent enzyme, methionine synthetase, in rats exposed to concentrations as low as 860 parts per million for 24 hours.<sup>7</sup>

Anaesthetists are continually exposed to nitrous oxide. Nunn *et al*, however, failed to find any evidence of abnormal methionine synthetase activity by measuring serum concentrations of methionine, valine, isoleucine, or leucine in 10 anaesthetists working in surgeries where scavenging was not used and the mean average concentration of nitrous oxide was 150-400 parts per million.<sup>8</sup> They concluded, therefore, that in these subjects

the methionine synthetase activity was normal. As they pointed out, however, methionine concentrations may be maintained despite depression of vitamin B<sub>12</sub> by either increased dietary intake or methylation of homocysteine by the betaine pathway. Other workers have also failed to find signs of interaction between vitamin B<sub>12</sub> and nitrous oxide in operating theatre staff.<sup>9</sup>

Dentists who use nitrous oxide as an analgesic during prolonged dental conservation procedures may be exposed to concentrations of up to 7000 parts per million,<sup>10</sup> and Layzer reported neurological changes, similar to those occurring in subacute combined degeneration of the cord, in 15 dentists exposed to nitrous oxide.<sup>11</sup> Although 13 later admitted abuse of the gas, two were using nitrous oxide in the conventional manner. We therefore investigated the possibility of bone marrow toxicity in dentists who habitually use nitrous oxide for long term relative analgesia. This technique, which is widely used, entails administering roughly 30% nitrous oxide in oxygen through a nasal mask for sedation and analgesia during dental conservation.<sup>12</sup>

## Methods

Twenty one dentists volunteered to take part in the trial. Each was given a full explanation of the study. Exposures to nitrous oxide were measured with an atmospheric sampler (Nitrox nitrous oxide dosimeter, Landauer), and one was supplied to each dentist with operating instructions.<sup>13</sup> The device, which was worn on the lapel, was uncapped at the start of each treatment with nitrous oxide and recapped at the end. Each dentist recorded the duration of his exposure to nitrous oxide, enabling an average weighted for time to be calculated.

The trial period varied from three to 11 weeks. During this time each subject was invited to this hospital, where he completed a questionnaire giving details of relevant medical history (for example, neurological disease and blood disorders), intake of alcohol, diet, and the use of any drugs. Individual records of exposure to nitrous oxide and details of any scavenging used during the sampling period were obtained. Each dentist was also asked in confidence whether he abused nitrous oxide. A neurological examination was carried out on each subject with particular regard to dysfunction of the dorsal column. Haematological investigations were carried out using standard techniques.<sup>14</sup> Full blood counts were obtained using a Coulter-S plus counter. A peripheral blood film was examined, a differential white cell count and a reticulocyte count performed, and the presence of neutrophils with hypersegmented nuclei noted. Serum vitamin B<sub>12</sub><sup>15</sup> and serum and red cell folate<sup>16</sup> concentrations were measured by microbiological assay. Finally, aspiration of sternal bone marrow was performed under local anaesthesia.

The bone marrow aspirate was examined morphologically and by the deoxyuridine suppression test, which provides an objective measure of synthesis of DNA dependent on vitamin B<sub>12</sub> and folic acid.<sup>17 18</sup> Briefly, the test consists of the short term culture in vitro of bone marrow cells with tritiated thymidine. The uptake of tritiated thymidine by cells in the control tubes, which lack deoxyuridine, represents 100% uptake. When normal bone marrow is incubated with deoxyuridine subsequent uptake of tritiated thymidine is suppressed to less than 5% of the uptake in the control tubes. In vitamin B<sub>12</sub> or folate deficiency, however, or under conditions that interfere with the activity of these vitamins, the utilisation of deoxyuridine is defective and an increased percentage of tritiated thymidine is taken up and incorporated into DNA. The peripheral blood and bone marrow films were examined independently by two observers (RJA and J A L Amess) without prior knowledge of the results of the deoxyuridine suppression tests.

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## Results

The table shows the mean exposures of the dentists to nitrous oxide in parts per million (range 159-4000 parts per million) and the reported average weekly durations of exposure in hours.

Exposures of dentists to nitrous oxide

Case No	Time weighted mean exposure (ppm)	Weekly exposure (h)	Total exposure (ppm.h)	Deoxyuridine suppression (%)
1	2400	10	24000	1.6
2	1600	7	11200	2.4
3	3800	10	38000	1.9
4	1900	6	11400	7.1*
5	2500	10	25000	5.0*
6	1800	27	48600	8.6*
7	1386	4	5544	2.2
8	159	5	770	1.9
9	3116	2	6232	1.6
10	500	2.5	1250	2.7
11	260	3.5	910	3.7
12	620	10	6200	1.8
13	949	4	3796	3.0
14	482	2.5	1205	2.5
15	1000	5	5000	2.2
16	4600	2	9200	2.0
17	800	12	9600	2.0
18	159	0.5	80	2.1
19	1062	4	4248	2.2
20	1768	3	5309	4.3
21	592	3.5	2072	

ppm = parts per million.

\*Denotes abnormal suppression.

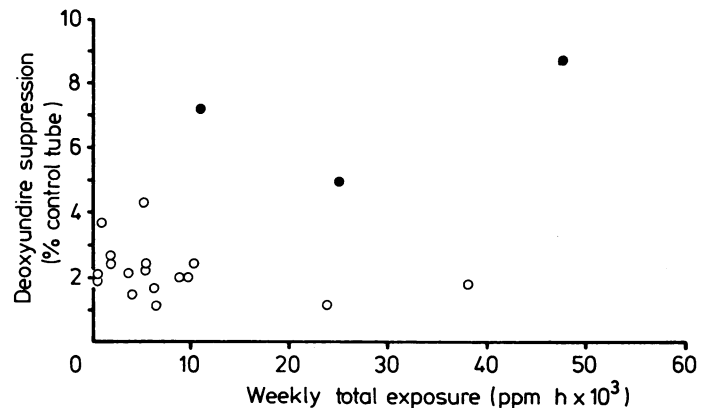
All the vitamin B<sub>12</sub> and folate concentrations were within the normal range, and no neurological abnormalities were detected.

Deoxyuridine suppression tests were performed in 20 of the 21 subjects. Seventeen of these yielded normal results. Definite abnormalities were found in two cases (8.6% and 7.1%), and a third yielded a value of 5.0%. This was greater than two standard deviations above the mean in a control group of 74 patients, who had a mean test result of 2.8 (SD 0.95)%.<sup>18</sup> All three of the dentists yielding abnormal results had normal full blood counts, with normal mean red cell volumes and no macrocytic red cells on the peripheral blood film. The two subjects in whom the deoxyuridine suppression test was definitely abnormal, however, had hypersegmented neutrophils: 6% and 9% of the peripheral blood neutrophils had five nuclear lobes (normal <5%) and, in the first subject, 1% of the neutrophils had six nuclear lobes. Furthermore, in both cases morphological abnormalities were also present in the bone marrow with easily seen giant metamyelocytes and mild megaloblastic change in the erythroid series. Both of these subjects consumed only minimal amounts of alcohol. Of the remaining subjects, three had a raised mean red cell volume (97, 99, and 100 fl), which may have been related to an increased intake of alcohol, and another had hypersegmented peripheral blood neutrophils (11% with five nuclear lobes), for which there was no cause other than exposure to nitrous oxide. Finally, minor non-specific morphological abnormalities, including occasional giant metamyelocytes, were present in a further 10 samples of bone marrow.

## Discussion

The deoxyuridine suppression test is a sensitive indicator of the disturbance in synthesis of DNA induced by nitrous oxide.<sup>8</sup> The abnormalities detected in this study, considered in conjunction with the morphological changes in the peripheral blood and bone marrow and normal vitamin B<sub>12</sub> and folate concentrations, suggest that these abnormalities in bone marrow are related to interference with the function of vitamin B<sub>12</sub> by nitrous oxide. Any link between the above haematological abnormalities and exposure to nitrous oxide will probably depend on both the duration of exposure to and the concentration of nitrous oxide. The concentration of nitrous oxide was, as we had expected, extremely high—over 10 times that experienced by anaesthetists in the study of Nunn *et al.*<sup>8</sup> Despite this none of the three dentists who had been exposed to the highest concentrations of nitrous oxide had any definite haematological abnormality.

However, when duration of exposure was included (that is, when the total weekly exposure was calculated from the concentration of nitrous oxide in parts per million and the duration of exposure) the dentists with the highest, third highest, and fifth highest exposures showed abnormal reactions to deoxyuridine suppression tests (figure).



Total weekly exposure of subjects to nitrous oxide related to results of deoxyuridine suppression test. ● = Abnormal suppression.

These results are in reasonable agreement with experimental animal work. Nunn *et al* found that methionine synthetase activity was inhibited by lower concentrations of nitrous oxide if the duration of exposure was increased, but they could find no evidence of significant inactivation of vitamin B<sub>12</sub> at concentrations of nitrous oxide under 1000 parts per million. The lowest concentration of nitrous oxide at which definite abnormalities occurred in our subjects was 1800 parts per million.

In summary, we have confirmed previous work showing that some dentists are exposed to extremely high levels of nitrous oxide. This study provides the first direct evidence that occupational exposure to nitrous oxide may cause depression of vitamin B<sub>12</sub> function and that this results in measurable changes in bone marrow secondary to impaired synthesis of DNA. In the United Kingdom at present there are no recommended safe upper limits for dentists and anaesthetists, and in the United States of America the upper limits recommended by the National Institute of Occupational Safety and Health are so low as to be practically unattainable.<sup>19</sup> We would recommend a concentration of 400 parts per million. This is attainable with effective scavenging<sup>10</sup> and is two to three times lower than the concentration at which appreciable depression of vitamin B<sub>12</sub> function has been detected, irrespective of duration of exposure, in animals or man.

We thank Dr J A L Amess for advice and the review of the peripheral blood and bone marrow films. RJA was supported by a Wellcome Trust pathology research fellowship, and ACP was supported by a grant to Dr J F Burman from the joint research board of St Bartholomew's Hospital. We particularly wish to thank the dentists who participated in this study.

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(Accepted 11 June 1985)

## Efficacy of feverfew as prophylactic treatment of migraine

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### Abstract

**Seventeen patients who ate fresh leaves of feverfew daily as prophylaxis against migraine participated in a double blind placebo controlled trial of the herb: eight patients received capsules containing freeze dried feverfew powder and nine placebo. Those who received placebo had a significant increase in the frequency and severity of headache, nausea, and vomiting with the emergence of untoward effects during the early months of treatment. The group given capsules of feverfew showed no change in the frequency or severity of symptoms of migraine. This provides evidence that feverfew taken prophylactically prevents attacks of migraine, and confirmatory studies are now indicated, preferably with a formulation controlled for sesquiterpene lactone content, in migraine sufferers who have never treated themselves with this herb.**

### Introduction

Feverfew (*Tanacetum parthenium*) is a medicinal herb commonly used in self treatment for conditions such as migraine and arthritis. In one survey more than 70% of 270 migraine sufferers who had eaten feverfew leaves every day for prolonged periods claimed that the herb decreased the frequency of the attacks or caused them to be less painful, or both.<sup>1</sup> Many of these people had failed to respond to orthodox medicines. Although possible mechanisms of action have been proposed for the beneficial effects of feverfew,<sup>2-4</sup> no one appears to have questioned whether it is in fact clinically effective in any condition.

From a large number of migraine sufferers known to the City of London Migraine Clinic to be treating themselves with feverfew 20 were invited to enter a double blind study, aware only that they might be treated with either active dried feverfew or placebo.

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### Patients and methods

We aimed to recruit 20 patients who had been treating themselves with raw feverfew leaves every day for at least three months. Ten patients were to receive active treatment consisting of freeze dried, powdered feverfew leaves in capsule form and 10 placebo. The patients had to have a history of common or classical migraine of at least two years' duration with no more than eight attacks a month at the time of admission.

We had intended to exclude patients who had taken the following drugs within one month of the study: tranquillisers,  $\alpha$  blockers,  $\beta$  blockers, antidepressants, non-steroidal anti-inflammatory agents, or clonidine and pizotifen used prophylactically. In the event we decided that this criterion could be waived if patients had begun prophylactic treatment with one of these drugs before starting to use feverfew daily, and provided that those taking antidepressants or tranquillisers showed no signs of current mental illness.

Before admission the nature and purpose of the study, including possible benefits and problems, were explained to each patient by one of us (ESJ), and the patient's informed consent to participate was obtained. Three patients refused to participate because they feared the possible consequences of being assigned to the group receiving placebo. The study was approved by the ethical committee of the City and Hackney District Health Authority.

**Trial design**—The trial was a double blind, placebo controlled comparison between two groups. Successive patients who had suffered from classical or common migraine for at least two years were allocated randomly to receive either feverfew or identical placebo capsules in numbered packs. Patients were instructed to take two capsules every morning with food for six periods of four weeks. They were instructed to treat acute attacks of migraine with soluble aspirin or their usual drug.

**Diary cards**—Patients were instructed how to record the various visual symptoms, nausea, vomiting, and headache (including times of onset and relief and any additional treatment) and to grade them according to severity on diary cards provided for each period. The severity of nausea or vomiting was recorded as: 0 = neither nausea nor vomiting; 1 = nausea only; 2 = vomiting, single episode; 3 = vomiting, repeated episodes. Headache was scored: 0 = no pain; 1 = mild, unpleasant but not affecting work or recreational activities; 2 = severe, reducing ability to work or carry out recreational activities; 3 = incapacitating, unable to work or carry out recreational activities; 1 = duration up to six hours; 2 = duration between six and 24 hours; 3 = duration greater than 24 hours. Presence of usual visual disturbance scored 1. Use of drugs was scored: 1 = use of repeated doses of minor analgesics; 1 = use of single dose of ergotamine; 2 = use of repeated doses of ergotamine. The cards were reviewed at intervals of one to two months throughout the study.

**Preparation of feverfew capsules and dosage**—Each freeze dried leaf containing five leaflets weighed a mean (SEM) of 25.7 (0.7) mg (n = 52). The mean daily dose of feverfew used by patients before entry to the study was 2.44 leaves (roughly 60 mg). We therefore decided that the dose of each capsule should be fixed at 25 mg and that each patient should receive two capsules daily. Preparation of the opaque capsules of hard gelatin (Eli Lilly, size 2) was supervised by DMH and PJH,