TITLE: DECREASED THYMIDINE PRODUCTION IN CELLS EXPOSED TO NITROUS OXIDE

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Introduction: Blackburn et al.[1] reported that exposure to high concentrations of nitrous oxide (N_2O) irreversibly oxidizes vitamin B_{12}, rendering it inactive as a coenzyme in certain biochemical reactions. A specific example is the decrease in deoxyuridine conversion to thymidine due to inhibition of the enzyme thymidylate synthetase (TS). This interference in DNA base production and in subsequent DNA synthesis is best observed in actively dividing cells; bone marrow and the rapidly developing fetus are prime targets. The present study investigates the dose-dependent effect of N_2O on inhibition of TS in pregnant rats using the deoxyuridine suppression (DS) test on bone marrow cells. Also investigated is the effect of exposure on fetal weights.

Methods: Forty-eight timed pregnant Sprague-Dawley rats, approximately 220 grams, were divided into four groups. On day 9 of gestation, they were exposed without food and water to either 0 (air), 0.75, 7.5, or 75.0% N_2O, for 24 hours. Immediately after exposure, half of the animals, six rats from each group, were killed by carbon dioxide, the abdomen opened and the number and weight of fetuses recorded. Femoral bone marrow was collected into cold Hank's solution and used for the DS test by the method of Wickramsinghe and Longland[2]. In brief, nucleated cells, suspended in serum, were incubated with and without deoxyuridine for 15 minutes, then for an additional hour in the presence of tritiated thymidine. The amount of tritium incorporated into DNA was counted. On day 3 after exposure, the remaining animals were killed and similarly examined.

Results: During exposure, rats in the 75% N_2O group were slightly somnolent. Otherwise, animals showed no obvious ill effects during or after exposure. Average number of fetuses per litter (approximately 12) and average fetal weights (approximately 250 mg) were not different among the groups. The results of the DS test, given as percentages of tritiated thymidine incorporated in deoxyuridine supplemented versus deoxyuridine starved cells, are given in the table below. On day 1 after exposure, there was a 2-fold increase in thymidine uptake in the 7.5 and 75% groups. Values returned to control levels by day 3.

Table: DS Test Results (Mean ± SEM, n=6)

% N_2O	% Thymidine Incorporated	
	Day 1	Day 3
0	16 ± 1	19 ± 3
0.75	16 ± 1	22 ± 4
7.5	32 ± 3*	17 ± 1
75.0	31 ± 1*	23 ± 5

*Significantly above air control ($p < 0.01$)

Discussion: Cells with inactive TS are unable to use the exogenous deoxyuridine, and therefore, take up increased amounts of tritiated thymidine. Failure to suppress thymidine uptake into maternal bone marrow cells on day 1 after exposure, indicates marked inhibition of TS by 7.5 and 75.0% N_2O. Although not directly measured, all other exposed cells including those of the fetus would be expected to be similarly affected. Such an interference to DNA synthesis could severely affect rapidly dividing fetal cells. Unfortunately, the present test is not an appropriate measure of TS activity in the fetus because bone marrow cannot be obtained from fetuses on days 10 or 12 of gestation. To determine TS activity in the fetus, we are assessing the possibility of performing the DS test with spleen cells taken from fetuses at a later stage of development. Ultimately one would want to correlate the biochemical changes, such as measured in this study, with morphological abnormalities in similarly exposed rat fetuses allowed to develop to term. Until more definitive studies can be performed, the present results provide the best evidence that the teratogenic effects seen with N_2O are the result of TS inactivity and the subsequent decrease in DNA base production and DNA synthesis.

References:

1. Blackburn R, Kyaw M: Reaction of Cob(I)alamine with Nitrous Oxide and Cob(III)alamine. Faraday Transactions 73:250-255, 1977.

2. Wickramsinghe SN, Longland JE: Assessment of Deoxyuridine Suppression Test in Diagnosis of Vitamin B_{12} or Folate Deficiency. BMJ 3:148-152, 1974.