Date

Title : NITROUS OXIDE DOES NOT ALTER SPERMATOGENESIS IN THE MOUSE

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The genetic toxicity of nitrous oxide (N_2O) has been suggested by epidemiologic surveys and by demonstrations of its teratologic activity in laboratory animals. In vivo mutagenesis assays did not evaluate the inhibitory effects of N_2O on cell division, which should be measured during an investigation of genetic toxicity in dividing cells. We have investigated N_2O mutagenesis following in vivo exposure with the mouse sperm morphology assay of Wyrobek and Bruce(1). To avoid artifact due to inhibition of cell division we measured the duration of spermatogenesis and the amounts of DNA produced following in vivo exposure.

Methods: Thirteen-week-old male mice of genotype (C57Bl/C3H)F_1 (Cumberland View Farms, Jackson, TN) were used in each experiment. The duration of spermatogenesis was measured following 4hr/da x 5 da exposure to 80%N_2O:20%O_2. Each of 30 mice received an intraperitoneal injection of 3H methyl thymidine, (3H-MT), 1μCi/g, immediately prior to the first exposure. Mice were sacrificed in groups of 5 daily on days 33-38 following the first day of exposure. The epididymides were removed, and each pair was lysed in 2ml Soluene 250. Radioactivity for each preparation was measured by scintillation counting, using 6ml Dimilume scintillation cocktail. A control group was similiarly prepared following exposure to air under identical conditions. For the analyses of sperm morphology and epididymal DNA, 5 groups of 6 mice each were used. Each group was exposed to a single concentration of N_2O(0, 10,20,40,80%) in N_2 + O_2 as needed to maintain FIO_2 = 20%. Exposures were 4 hr/day x 5 da. All animals were sacrificed 35 da following the first day of exposure, and the epididymides were removed. One epididymis from each mouse was used to measure DNA, and one was used for analysis of sperm morphology. Epididymal DNA was extracted using the method of Munro and Fleck(2) and quantitated fluorometrically using the method of Giles and Myers.(3)

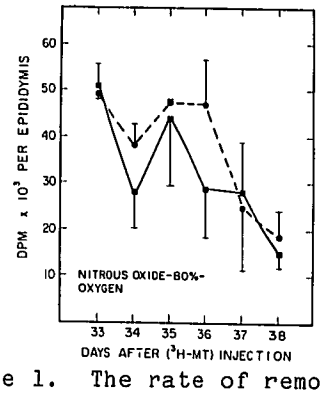

Figure 1. The rate of removal of 3H-MT from the epididymides following exposure to 80% N_2O (■—■) is not different from that found in the control group exposed to air (●---●).

TABLE 1. PER CENT ABNORMAL SPERM FOLLOWING N_2O EXPOSURE.

CONC. N_2O	% ABN. SPERM (± SEM)
0 (AIR)	1.07 ± .07
10	1.16 ± .06
20	1.21 ± .03
40	1.06 ± .06
80	1.22 ± .11

Results: Exposure to 80% N_2O:20%O_2 4hr/da x 5 da did not effect the pattern of 3H-MT removal from the epididymides (Fig. 1). Exposure to N_2O at 10,20,40 and 80% 4hr/da x 5 da did not change the amount of DNA present in the epididymis at the end of a normal 35 da cycle of spermatogenesis (Fig. 2). No increase of the percent abnormal sperm was found following exposure to any concentration of N_2O (Table 1).

Discussion: Even when the acute cytotoxic effects of N_2O are accounted for, we could find no evidence of genetic toxicity at any concentration with the sperm morphology assay. These data support the results of in vitro mutagenesis assays. They suggest that N_2O is not a mutagen. The reproductive toxicity ascribed to this agent does not appear to result from genetic damage.

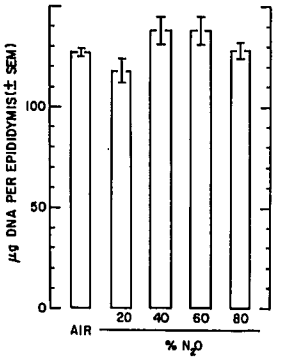

Figure 2. Epididymal DNA 35 days following the first day of exposure to N_2O.

REFERENCES:

1. Wyrobek AJ, Bruce WR. Proc Natl Acad Sci USA 72:4425-4429, 1978.
2. Munro HN, Fleck A. in Methods of Biochemical Analysis XIV:113-176, 1966.
3. Giles KW, Myers A. Nature 206:93, 1965.