Effects of Low Concentrations of Nitrous Oxide on Rat Pregnancy

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Pregnant rats were exposed to concentrations of nitrous oxide similar to those present in the operating room during routine occupational exposure for various periods during pregnancy. Those exposed to 15,000 ppm and 1,000 ppm for 24 hours/day had fetal death rates and pregnancies/rat ratios which were significantly different from the controls. Two groups exposed to 1,000 ppm for 8 hours/day also had fetal death rates significantly different from the controls. Exposure for 8 hours/day did not significantly alter the pregnancies/rat ratios. Differences in the fetal death rates between groups exposed from 6 AM to 2 PM and from 2 PM to 10 PM suggest a diurnal variation in the embryotoxic effect of nitrous oxide in the rat. (Key words: Nitrous oxide; Toxicity; Occupational disease.)

Since its introduction as an anesthetic in 1844, nitrous oxide has accumulated an impressive record of clinical safety. Chronic exposure to 40–50% nitrous oxide for several days, however, causes bone marrow depression in man¹ and is embryotoxic and teratogenic to the rat.² Recent surveys have shown that operating room personnel have a higher rate of spontaneous miscarriage than the general population.³ In order to find out whether chronic administration of low concentrations of nitrous oxide is embryotoxic to the rat, we exposed pregnant rats to concentrations of nitrous oxide similar to those found in the operating room environment for various periods of time during pregnancy and then sacrificed the animals to determine fetal death rates for exposed and control groups.

We found that nitrous oxide was present in concentrations ranging from 330 to 9,700 ppm in the inhalational zone of the anesthesiologist during routine conditions.⁴ Concentrations were usually in the neighborhood of 1,000 ppm. The higher values occurred when the anesthesiologist and anesthesia machine were partially enclosed in a tent of surgical drapes and high flow rates were used. Concentrations ranged from 310 to 550 ppm in the inhalational zone of the surgeons.

Materials and Methods

All groups of rats were obtained from the Simmons Laboratories, Gilroy, California. Pregnant rats mated at the same time were ordered. Groups 1–4 were exposed in chambers constructed from metal shelving and plastic sheets. Rat cages were placed on the shelves and the plastic sheets secured to make the chamber as tight as possible. Groups 5–10 were exposed in environmental exposure chambers manufactured by Germ Free Laboratories, Miami, Florida. All gases used were ordered mixed at the desired concentrations and delivered in “H” tanks from the Ohio Chemical Company. Flow rate through the exposure chambers was 5 l/min. Groups 1 and 3 were exposed to 15,000 ppm and 1,000 ppm N₂O and 22 per cent O₂ and balance nitrogen for 24 hours per day from days 8 to 13 and days 12 to 19 of pregnancy, respectively. Groups 2 and 4 were control groups exposed to 22 per cent O₂ and 78 per cent N₂ for 24 hours per day from days 8 to 13 and days 12 to 19 of pregnancy, respectively. Groups 5–10 were exposed to either 1,000 or 100 ppm nitrous oxide in 22 per cent O₂ with balance N₂ for 8 hours per day from days 10 to 13, days 14 to 19, or days 10 to 19 of pregnancy. Group 11 was a control group exposed to compressed air.

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### Table 1. Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Pregnant Rates</th>
<th>Number of Pregnancies</th>
<th>N2O Concentration (ppm)</th>
<th>Time Exposed (Hours/Day)</th>
<th>Days of Pregnancy Exposed</th>
<th>Implantations/Rat</th>
<th>Fetal Death Rate</th>
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<tr>
<td>1</td>
<td>12</td>
<td>72</td>
<td>15,000</td>
<td>24</td>
<td>8-13</td>
<td>6.0 $P &lt; 0.005$</td>
<td>11.1 $P &lt; 0.005$</td>
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<tr>
<td>2</td>
<td>10</td>
<td>112</td>
<td>0*</td>
<td>24</td>
<td>8-13</td>
<td>11.2</td>
<td>1.8</td>
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<tr>
<td>3</td>
<td>6</td>
<td>53</td>
<td>1,000</td>
<td>24</td>
<td>12-19</td>
<td>8.8 $P &lt; 0.05$</td>
<td>18.9 $P &lt; 0.05$</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>100</td>
<td>0*</td>
<td>24</td>
<td>12-19</td>
<td>11.1</td>
<td>4.0</td>
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<tr>
<td>5</td>
<td>10</td>
<td>109</td>
<td>1,000</td>
<td>S</td>
<td>10-13</td>
<td>10.9</td>
<td>18.4 $P &lt; 0.01$</td>
</tr>
<tr>
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<td>9</td>
<td>97</td>
<td>100</td>
<td>S</td>
<td>10-13</td>
<td>10.8</td>
<td>15.5 $P &lt; 0.05$</td>
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<td>7</td>
<td>7</td>
<td>76</td>
<td>1,000</td>
<td>S</td>
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<td>10.9</td>
<td>14.5 $P &lt; 0.05$</td>
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<tr>
<td>8</td>
<td>10</td>
<td>99</td>
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<td>S</td>
<td>14-19</td>
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<tr>
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<td>0†</td>
<td></td>
<td></td>
<td>10.2</td>
<td>5.4</td>
</tr>
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</table>

* Exposed to 22 per cent O2, 78 per cent N2.
† Exposed to room air.
‡ Groups 9 and 10 were exposed from 2 PM until 10 PM; Groups 5-8 were exposed from 6 AM until 2 PM.
§ Significant when compared with Group 2 only.

Nitrous oxide concentrations were measured twice daily using a Beckman GC 72-5 gas chromatograph equipped with a helium ionization detector. Concentrations of nitrous oxide were constant at the desired level except in Group 1. Due to leakage from the homemade chamber, concentrations were usually lower than designated, ranging between 9,000 and 12,000 ppm, with a maximum of 15,000 ppm and a minimum of 100 ppm.

Oxygen concentrations, measured twice daily using a Beckman paramagnetic oxygen analyzer, were always between 21 and 22 per cent. Soda lime was spread on the floors of the chambers and changed daily. CO2 concentrations were measured daily using a Beckman CO2 infrared analyzer. Concentrations did not exceed 0.4 per cent and generally were 0.1 per cent or lower.

Temperatures in the chambers ranged from 77 to 80 F for Groups 1-4 and from 74 to 78 F for Groups 5-11.

After removal from the exposure chambers, the animals were kept in cages until day 20 of the 21-day pregnancy, when they were sacrificed and examined. Implantation in the rat occurs on day 5 of pregnancy. Lethal effects manifested shortly after implantation may result in total resorption of the conceptus by day 20, leaving no evidence of pregnancy. Therefore, a pregnancy per rat ratio was calculated for each group. Evidence of fetal death was sought by first examining the intact uteri and counting resorption spots, then incising them and noting fetal deaths manifested by macerated or dead fetuses. We use the term “fetal death” to include resorption spots, macerated fetuses, and dead fetuses.

### Results

The results are listed in table 1. The non-uniform numbers of rats in the groups resulted from some animals not being pregnant.

The pregnancy per rat ratios for Groups 1 and 3 (6.0 and 8.8) differed significantly from controls (11.2 and 11.1), with $P < 0.005$ and $P < 0.05$, respectively, using the Wilcoxon rank sum test. The pregnancy per rat ratios for Groups 5-10 were not significantly different from controls. These data suggest that 24 hour/day exposure causes early fetal death with total resorption of the conceptus by day 20 of the pregnancy. This was not seen with the 8 hour/day exposures. Fetal death rates for both 24 hour/day exposures (11.1 and 18.9 per cent for Groups 1 and 3) were significantly
different from controls (1.8 and 4.0 per cent for Groups 2 and 4), with $P < 0.005$ and $P < 0.05$, respectively.

For statistical analysis of fetal death rates in Groups 5–11, analysis of variance was followed by Scheffe’s multiple-comparisons procedure of the log of $x + \frac{1}{2}$, where $x$ was the number of fetal deaths for an individual rat. This is equivalent to an analysis of fetal death rates, since the litter sizes did not vary significantly. Three rats were excluded from the analysis of fetal deaths because they had three or fewer implantations, and thus gave almost no information about fetal death rates. Only litters of seven or more offspring were considered. Rats exposed to 1,000 ppm nitrous oxide for 8 hours/day from 6 AM to 2 PM (Groups 5 and 7) also had fetal death rates (18.4 and 14.5 per cent) which were significantly different from control (5.4 per cent), with $P < 0.01$ and $P < 0.05$, respectively. Interestingly, Group 9, exposed to 1,000 ppm nitrous oxide later in the day (2 PM to 10 PM) for the longest duration (days 10–19), had a fetal death rate (7.3%) only slightly higher than the control groups, suggesting diurnal variation in the embryotoxic effects of nitrous oxide. Of the 100-ppm exposures, only Group 6 had a fetal death rate higher than the control. Considering litters of seven or more only, the Group 6 fetal death rate was significantly different from that of Group 2 only, with $P < 0.05$.

Discussion

There are numerous reports of toxicity due to acute exposure to anesthetic concentrations of inhalation agents. We have demonstrated embryotoxicity in the rat due to chronic exposure to low concentrations of nitrous oxide. This finding is significant since it is the first study, to our knowledge, which demonstrates toxic effects of an anesthetic agent due to chronic exposure to low concentrations.

The mechanism through which this fetal toxicity in rats manifests itself is unknown. The diurnal variation in the embryolethal effects of nitrous oxide is of interest. We are unable to explain this effect. The possibility of diurnal variation in the rate of growth of fetal rats warrants investigation.

We must be cautious in interpreting this data in relation to human pregnancy. This study demonstrates the need for extensive testing of chronic exposure to low concentrations of inhalation anesthetic agents for possible embryotoxic, teratogenic, mutagenic and carcinogenic effects.

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References