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The effects of intermittent chronic exposure of nitrous oxide on rat fertility and pregnancy

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Abstract

High incidences of cancer, hepatic disease, renal disease, spontaneous abortion, premature delivery, and congenital deformities in their offspring have been reported amongst operation-room personnel. These conditions have been attributed to their exposure to anesthetic gases. Dentists who are exposed to anesthetic gases are also reported to have high incidences of these diseases. While nitrous oxide is the only anesthetic gas to which most dentists are exposed, they are exposed to high concentrations because of patients' open mouths, recirculating air conditioning and high flow rates. Wistar-Lewis inbred rats were exposed to 70 percent nitrous oxide and oxygen two hours each day for 30 days. After the thirtieth day, various matings were made between experimental and control animals. The results of these matings showed that this chronic intermittent dose had no effect on their reproductive capacity or on their offspring. A histologic examination of the gonads of the rats exposed to nitrous oxide failed to show microscopic changes reported by other investigators when rats were continuously exposed.

Introduction

The occurrence of occupational hazards in dentistry has been recognized for many years. Early in the profession's history, indiscriminate use of X-radiation proved hazardous for the dentist as well as the patient. Recently, much has been written about the improper handling of mercury and mercury contamination of the dental operatory. It is the purpose of this investigation to examine nitrous oxide which, because of its popularity and widespread use, may prove hazardous to those who are continually exposed.

Literature Review

Occupational hazards have been demonstrated by many investigators to occur in the operating-room environment. In 1965, Vaisman[1] reported an unusually high incidence of headache, fatigue, nausea and pruritis in the 303 Russian anesthesiologists surveyed. In addition, among the female anesthesiologists, 18 of the 31 pregnancies that occurred during the study period ended in spontaneous abortion. There were two premature deliveries and one child was born with an unspecified congenital abnormality. Other studies have noted higher incidences of cancer, hepatic and kidney disease in addition to spontaneous abortions, premature delivery and congenital deformities in the children of operating personnel. Exposure to anesthetic gases seems to be the most likely etiologic factor.[1,2,3,4,5,6,7,8,9,10,11]

One of the inhalation anesthetics, nitrous oxide, has been demonstrated to have many toxic side effects. In 1956, Lassen[12] studied the effect of 50 percent nitrous oxide on human bone marrow. He found a profound depression of this tissue when exposure was for a prolonged period during treatment of tetanus and poliomyelitis. Later in a study by Parbrook[13] in 1969,

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it was reported that nitrous oxide has a depressive effect on the rat's hemopoetic system. Green[14] demonstrated that the RNA/DNA ratio of the rat hemopoetic organs, the bone marrow and the thymus, were markedly altered by the administration of nitrous oxide. He found that DNA content decreased while RNA content increased or stayed the same. Green[15] found that the leukopenic effect was strain specific in rats.

With the toxic effects on the hemopoetic system firmly established, researchers looked to other tissues with rapid cell division. Snegireff and others[16,17] have shown nitrous oxide to be lethal to the developing chick embryo, but no congenital abnormalities could be demonstrated in their studies. Fink and Shepard[18,19] demonstrated teratogenic effects of nitrous oxide in Sprague-Dawley rats. These were primarily skeletal changes. Fetal resorptions (similar to spontaneous abortions in man), decreased birth weight, and decreased crown-rump length have been demonstrated by Bussard and associates[20,21] when pregnant hamsters were exposed to a combination of halothane and nitrous oxide. Corbett[22] has shown embryo toxicity in rats at levels as low as 0.1% or 1000 ppm. An alteration of the time of day of exposure was also shown to influence the number of fetal deaths that occurred in this study.

Kripke and his associates (1976)[23] have shown that nitrous oxide is toxic to another rapidly dividing system, spermatogenesis. Degeneration and necrosis have been reported in cells undergoing division and maturation while the vasculature, resting spermatogonia and the cells of Sertoli and Leydig were resistant. Not only was there a decrease in quantity of spermatozoa and dried weight of the testes, but bizarre cells, such as spermatozoon with two heads or two tails could be demonstrated. Animals exposed intermittently were less affected than those who were exposed continuously to the 20 percent atmosphere of nitrous oxide over the 35-day test period. If sufficient time elapsed after the cessation of exposure to nitrous oxide, recovery was evident.

It appears that nitrous oxide can affect mitosis and the DNA-synthetic phase(s) of the cell cycle. Brinkley[24] has reported that nitrous oxide causes misalignment of chromosomes on the spindle and hence a typical mitoses. This work supports the earlier report by Green[14] that nitrous oxide decreases DNA synthesis in rat bone marrow and thymus.

Epidemiologic surveys are yet another source of information available which suggests dangers of being exposed to trace contaminations of anesthetic gases.

Askrog and Harvold (1970)[2] studied a group of female anesthetists and operating-room nurses. They showed that the percentage of spontaneous abortions in these individuals rose from about 10 percent before employment to 20 percent after employment. In addition, the group showed a significant increase in premature delivery as well as an increase in the number of female births.

Cohen, Bellville, and Brown (1971)[4] found a similar relationship between spontaneous abortion and exposure to the operating room. Spontaneous abortions among anesthetists occurred in nearly 40 percent of the pregnancies. Operating-room nurses had a 30 percent rate as compared to control groups which had approximately a 10 percent rate of spontaneous abortions. Corbett *et al.* (1974)[13] showed that nurse-anesthetists who worked during their pregnancies had a higher incidence of birth defects (16.4 percent) in their offspring than those who did not work (5.7 percent).

A 1968 study by Bruce and others[3] showed that anesthesiologists had a somewhat lower than average rate of lung cancer and coronary artery disease, but much higher rates of suicide and lymphoid as well as reticuloendothelial cancers.

Corbett and his co-workers[8] surveyed a group of Michigan nurse-anesthetists. Of the 621 surveyed, 84.5 percent responded. A total of 33 malignancies were reported. The expected rate for this group, adjusted for sex and age distribution, is 402.8 cancers per 100,000. The incidence among this group is 1,333.3 per 100,000, over three times as many tumors.

A national study of occupational disease among operating-room personnel was conducted by the American Society of Anesthesiologists (Cohen et al., 1974).[5] The results of their survey indicate that the female members of the operating-room team were subject to increased risks of spontaneous abortion, congenital abnormalities in their children, cancer, renal and hepatic disease. No increased rate of cancer or renal disease was seen among the male operating-room personnel, but a similar increase in the incidence of hepatic disease could be demonstrated. An increased risk of congenital abnormalities was also present in the children of the male operating-room personnel.

A similar mail survey was undertaken by Cohen and the American Dental Association.[6] A slightly increased incidence of congenital abnormalities present in the children of those in the exposed group and the greatly increased incidence of liver disease are similar in magnitude to the male anesthesiologists in the ASA survey. However, two points differ from the physician's survey. First, there was a slight increase in cancer rate in the exposed dentists. Secondly, a significant increase in spontaneous abortions was found in the unexposed wives of exposed dentists.

It is clear that nitrous oxide is not the only anesthetic contaminant to which participants in the ASA and ADA surveys were exposed. Generally, nitrous oxide is the only anesthetic gas to which the dental practitioner (other than an oral surgeon) is exposed. It is unclear how these two surveys relate to the general dentist or the pedodontist.[25,26,27,28,29] It has been noted by Sturrock[30] that there is a synergistic relationship between halothane and nitrous oxide in the production of nuclear abnormalities in a culture of dividing fibroblasts.

Exposure of the dentist to anesthetic waste gas is reported by Millard[31] in 1974 to be as high as 6767 ppm nitrous oxide after one hour of an operative procedure. Campbell (1977)[32] reported levels of nitrous oxide as high as 90,000 ppm four inches in front of the pop-valve. The exposure to waste gases can be related to the time of exposure, flow of gases, the difficulty of the procedure, the circulation of the room, as well as the size of the room. Whitcher[33] in 1975 reported levels in the surgeons' breathing zone in the operating room to vary between 310-550 ppm.

Unlike hospital operating rooms, most dental operatories are air conditioned with recirculating units. This, compounded with an open-mouthed patient and the use of high-flow rates of anesthetic gases, leaves the dentist at high risk.

A brief comment should be made about the data presented above. One should be aware of the dangers in making direct correlations between animal studies and the conditions in humans. This is especially true when data from inbred animals, those with no heterozygosity, are extrapolated to a human population with a great variety of genetic variability. The second type of data available to test the effects of anesthetic gases is from epidemiologic surveys.

These data must be evaluated on their own merits, but one important source of bias is the percentage of questionnaires returned in that survey. Rates vary considerably. The large scale ADA survey[6] had a return rate of 38.9 percent for general dentists. On the other hand, Corbett's[8] study of nurse-anesthetists had a return rate of 84.5 percent. With these data, one should be very careful of low return rates where those who had something to communicate or a point to make may be more likely to reply.

Cohen's[5,6] studies of the members of the ASA and ADA raise serious questions as to the effect of chronic exposure to anesthetic gases on the reproductive system. Although nitrous oxide was not the only anesthetic contaminant participants of these two studies were exposed to, it is the primary agent used by most dentists. The purpose of this investigation is to reproduce some of the effects found in Cohen's studies in experimental animals under controlled conditions using nitrous oxide as the only anesthetic agent.

Methods and Materials

Seventy-eight Wistar-Lewis inbred albino rats (Charles Rivers Laboratory) were obtained at 39 days of age. Upon arrival, the 39 males and 39 females were immediately randomly assigned to various groups seen in Table 1.

Table 1.

Group	Number of Animals
A. Males exposed to N_2O/O_2	22
B. Males exposed to O_2 only	12
C. Males exposed to no gases	5
D. Females exposed to N_2O/O_2	22
E. Females exposed to O_2 only	12
F. Females exposed to no gases	5
Total Animals	78

From these six groups, three experimental and two control pairings were made as shown in Table 2. The remaining nine males and nine females were used for the histologic section of this investigation.

Table 2.

Pairings	Groups	Number of Animals
1	A and D	14
2	A and E	12
3	B and D	12
4	B and E	12
5	C and F	10
	Total Animals	60

After the animals were placed in their cages, a color code was affixed to the cage to designate to which group they belonged. They were housed in wire mesh cages, ate a diet of Purina laboratory chow, and drank tap water *ad lib.* Temperature, humidity and light cycle were electronically controlled throughout the investigation. The animals were given a 10-day period to recover and adjust to their new surroundings.

The exposure to gases was done in a chamber constructed of 1/8-inch Lucite transparent plastic, measuring 6 1/2 x 19 x 36 inches. The connections were made of standard disposable anesthesia hosing. The system was open; no CO_2 absorption nor rebreathing system was used. Excess gases were vented to the outside. A recently calibrated anesthesia machine was used to deliver the gases from standard "G" tanks.

Each group that was to be exposed to gases was removed from its cage, placed in the exposure chamber, and moved out of the vivarium. The gases ran for two hours. At the end of the exposure, the chamber containing the animals was moved back into the vivarium and the animals were returned to the appropriately labeled cage. No provision was made for food or water during the time of exposure to gases. Animals exposed to oxygen only were exposed to 100 percent O_2 (10 liters/minute flow) for two hours each day for 30 days. Animals exposed to nitrous oxide were exposed to 70 percent N_2O (seven liters/minute flow) and 30 percent O_2 (three liters/minute flow) for two hours each day for 30 consecutive days. Exposures were conducted randomly during the day, and care was taken to maintain the appropriate day/night condition.

Because there were only twenty-four animals exposed to oxygen alone, the males and females were exposed together. They were separated by a screen which allowed free airflow between the two compartments but did not allow contact between males and females. Because of the large number of animals exposed to nitrous oxide and oxygen, it was necessary to expose the males and females separately.

After five, 10, 20, and 30 days of exposure, two males and two females in the N_2O/O_2 groups, selected at random, were sacrificed by an intraperitoneal injection of a lethal dose of sodium pentathal. Sacrifices were conducted immediately following exposure. After death was confirmed, the animal was examined. The gonads were dissected out and stored in 10 percent formalin to prepare for microscopic examination. To enable better fixation, the testes were cut while the ovaries were left whole. One male and one female were selected from the N_2O/O_2 groups (A and D) before exposure began to serve as controls for the histologic section of the investigation. Several sections were made from each teste and ovary. They were stained with hemotoxylin and eosin and compared to the control for changes in the tissue.

Table 3. The number of litters that were born to each pairing with no significant difference in the number of litters each pairing produced

Pairing No.	Gas Exposure Female	Gas Exposure Male	No.of Pairings Producing Litters	No. of Pairings Producing no Litters	No. of Pairings
1	N_2O	N_2O	5	2	7
2	O_2	N_2O	4	2	6
3	N_2O	O_2	4	2	6
4	O_2	O_2	5	1	6
5	No Gas	No Gas	4	1	5
	Total Number of Pairings		22	8	30

$X^2 = .7051$
$p = NS$

Immediately following the thirtieth day of exposure to gases, the appropriate pairings were made as listed in Table 2. No attempt was made to confirm copulation or pregnancy. After 11 days, the females were placed in plastic maternity cages which had been previously numbered from one to 30. This insured the observations would be done without bias.

The number of pups in each litter was recorded soon after birth. In addition, the observer noted the number of live and dead pups at this time. Any gross deformities were noted. Twenty-six days later the first litter was sacrificed using ether. They were sexed, weighed and measured. As each litter became 26 days old, the same procedure was carried out.

Results

A total of 22 litters were born to 30 dams, distributed almost evenly amongst the pairings (Table 3). All births appeared uncomplicated and normal. No gross developmental deformities, such as cleft lip or cleft palate, polydactyly or syndactyly, missing limbs or other such gross changes, were noted in either experimental or control animals. The chi square test shows that there is no significant difference in the number of litters produced by the exposed and unexposed pairings. This suggests that the agents and experimental conditions had no effect on fertility.

Table 4 presents the number of pups and their sex, that were born to each pairing. There is no statistical difference either in the number or the sex distribution of the pups between the pairings.

The mean and standard error for weight and length of pups for each pairing is shown in Table 5. When this data is treated with the one-way analysis of variance and the Student-Newman-Keuls multiple range test, significant differences were detected between certain pairings (Tables 6 and 7). There is considerable overlap of the subgroups in the weight of pups (Table 6), but a more distinct difference in the ranges of the subgroups is seen in the length of the pups (Table 7). As shown in these tables, pairing three (females exposed to N_2O and males to O_2) and pairing five (no gas for males and females) showed no difference in weight or length of pups. Histologic examination of the ovaries and testes showed no demonstrable difference between experimental and control specimens, no focal or generalized destruction was noted in the testes or ovaries of experimental animals even when exposure to nitrous oxide was for 30 days.

It was decided to let the pups be born rather than sacrifice the mothers one or two days before delivery. Both methods have advantages and disadvantages. The decision was based on ease of the procedure but this meant an investigator had to be present when birth occurred because of possible cannibalism on the

Table 4. The number of pups and their sex born to each pairing with no significant difference observed in the number of pups born of the sex distribution

Pairing No.	Number of Pups	Female Pups	Male Pups
1	65	31	34
2	52	26	26
3	38	23	15
4	55	31	24
5	48	22	26
Totals	258	133	125
Total Number of Pups in Each Pairing	$X^2 = 7.54$ $p = 0.1$, NS		
Sex Distribution in Each Pairing	$X^2 = 2.802$ $p = 0.5$, NS		

Table 5. The weights and lengths of the pups born to each pairing with data divided into male, female and overall groupings

No. of Pups in Each Litter			Females Weight		Males Weight		Overall Weight		Females Length		Males Length		Overall Length	
			gm		gm		gm		mm		mm		mm	
Pairing No.	Females	Males	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	31	34	48.28	1.06	50.21	0.93	49.28	0.71	49.48	0.43	41.16	0.27	50.36	0.27
2	26	26	45.32	1.11	46.97	1.15	46.14	0.80	49.51	0.53	50.73	0.49	50.12	0.37
3	23	15	53.02	2.76	57.66	2.97	54.85	2.07	51.87	0.98	53.20	1.13	52.40	0.75
4	31	24	46.65	0.92	47.72	0.96	47.11	0.67	49.67	0.48	50.45	0.45	50.01	0.33
5	22	26	50.31	0.68	52.86	0.71	51.69	0.52	52.23	0.29	53.46	0.25	52.90	0.21

Table 6. The weight (in grams) of the male, female and combined pups ranked by mean weight*

One-Way Analysis of Variance Multiple Range Test (Student-Newman-Keuls)					
Weight of Female Pups					
Pairing No.	2	4	1	5	3
Mean	45.32	46.65	48.28	50.31	53.02
n	26	31	31	22	23
Weight of Male Pups					
Pairing No.	2	4	1	5	3
Mean	46.97	47.72	50.21	52.86	57.66
n	26	24	34	26	15
Combined Weights of Pups					
Pairing No.	2	4	1	5	3
Mean	46.14	47.11	49.28	51.69	54.85
n	52	55	65	48	33

* The one-way analysis of variance was applied to find significant differences. Underlined groups are not significantly different when the Student-Newman-Keuls multiple range test is applied.

Table 7. The length of the male, female and combined pups ranked by mean length*

	Length of Female Pups				
Pairing No.	1	2	4	3	5
Mean	49.48	49.51	49.67	51.87	52.23
n	31	26	31	23	22
	Length of Male Pups				
Pairing No.	4	2	1	3	5
Mean	50.45	50.73	51.16	53.20	53.46
n	24	26	34	15	26
	Combined Length of Pups				
Pairing No.	4	2	1	3	5
Mean	50.01	50.12	50.36	52.40	52.90
n	55	52	65	38	48

* Crown-rump length in millimeters.

The one-way analysis of variance was applied to find significant differences. Groups underlined are not significantly different when the Student-Newman-Keuls multiple range test is applied.

part of the mother. The more sophisticated method yields more information, but involves confirming copulation and timing the sacrifice accordingly. One must assume the agent in question does not affect the gestation period substantially. When the uterus is opened the number of viable fetuses, resorptions and non-fertilized sites can be counted.

The purpose of this experiment was to use well controlled circumstances to reproduce key findings in the ADA and ASA studies.[5,6] Because all pairings had no statistically different numbers of litters or pups, it appears that our methods were adequate. But, had the results differed and the pairings had statistically different numbers of litters or births in their litters, we could not have determined whether this was because of non-fertilization or resorptions (spontaneous abortions).

Discussion

Several animal studies have demonstrated that nitrous oxide has a toxic effect on embryos in pregnant animals.[18,20,22] Some epidemiologic surveys indicate that this effect is seen in humans also.[1,2,4,5,6,7,11] The ASA[5] and ADA[6] surveys conducted by Cohen seem to point to another toxic effect of anesthetic gases which may occur before pregnancy. In the ASA survey, an increased number of congenital anomalies occurred among the children of exposed male operating-room personnel. In the ADA survey, the wives of dentists who were exposed to nitrous oxide were found to be at higher risk for spontaneous abortions than the wives of unexposed dentists. In neither case was the embryo exposed to nitrous oxide. This investigation was an effort to test, under controlled conditions, whether some of these findings could be produced in animals.

No birth defects were detected in the experimental or control litters. No differences are seen in the num-

ber of litters, the size of litters or the sex distribution of the litters. Although significant differences exist in the mean length and weight of pups, an interpretation of the results is difficult. When the Student-Newman-Keuls multiple range test is applied to mean weight, considerable overlap of the subgroups is apparent. Although there are definite differences, it is this overlap that complicates the interpretation. A more definite difference is detected when the same test is applied to the length of the pups in the various pairings. The pairing exposed to no gas and those where the males were exposed to O_2 and the females to N_2O, were significantly different from the other pairings. Since the two control groups, those exposed to no gas and those where the males and females were exposed to O_2 only, fell into different subgroups, interpretation is again difficult. This strongly suggests that although there were statistical differences in weight and length, they were not indicative of the effects of experimental conditions. These differences may have been generated by the numbers of pups and the sex distribution in each litter.

The histologic section of this investigation supports the findings in the previous section. Differences in the control and test groups were not seen. Histologic study of the testes, failed to show the pathologic changes described by Kripke[23] when male rats were continuously exposed to nitrous oxide over a similar period of time.

The gross and histologic observations from this study showed nitrous oxide to have no effect on the reproductive capacity or on the offspring of the male and/or female rats, under these experimental conditions. Although extrapolation from animal studies to humans is always difficult, it is possible that intermittent exposure only to nitrous oxide, may not result in the toxic effects previously ascribed to other anesthetic gases.

Conclusion

Wistar-Lewis albino rats exposed to 70 percent nitrous oxide and 30 percent oxygen at 10 liters per minute flow for 30 days in the described container has no effect on:

1. Fertility — the number of litters/pairing
2. Reproduction — pups/litter
3. Offspring
   a. male/female ratio
   b. length
   c. weight
   d. gross deformities
4. Microscopic changes of their gonads

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