

Laboratory Report

Fetal Changes in Hamsters Anesthetized with Nitrous Oxide and Halothane

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Pregnant hamsters were exposed to 60 per cent nitrous oxide plus 0.6 per cent halothane from 9:00 A.M. to 12:00 noon on the ninth, tenth or eleventh day of gestation. Compared with control hamsters, the number of resorptions (similar to spontaneous abortion in man) was increased ($P < 0.05$) in those anesthetized on day 11 only. Mean fetal weight ($P < 0.001$, days 10 and 11) and crown-rump length ($P < 0.001$, day 10; $P < 0.02$, day 11) were decreased. The ratios of female fetuses to total surviving fetuses were similar in the control and anesthetized hamsters. These changes support results of previous studies suggesting that elective administration of inhalation anesthetics be avoided during early pregnancy in man. (Key words: Toxicity; teratogenicity; Anesthetics, volatile; halothane; Anesthetics, gases; nitrous oxide; Pregnancy; anesthetic embryotoxicity.)

IT HAS BEEN ESTIMATED that 50,000 women in the United States may require anesthesia and operation during gestation.¹ At present the inhalation anesthesia likely to be used in such a situation would be nitrous oxide-halothane in oxygen. Both nitrous oxide and halothane when administered individually in anesthetic concentrations to animals during early pregnancy were embryotoxic and/or teratogenic.^{2,3} It is possible that the combina-

tion of nitrous oxide and halothane produces different effects.⁴ This study reports the incidence of fetal resorptions and determinations of fetal size and sex following exposure of pregnant hamsters to anesthetic concentrations of nitrous oxide plus halothane in oxygen for three hours on the ninth, tenth, or eleventh day of gestation.

Methods

Virgin female hamsters (*Mesocricetus auratus*) were placed with males at approximately 10:00 P.M. Pregnancy was assumed when copulation was observed, followed by the presence of vaginal plugs. The 24-hour period immediately following copulation was considered day 1. The pregnant females were housed separately and were randomly assigned to one of three experimental or control groups.

The experimental hamsters were placed in a sealed 40-litre rectangular glass chamber for three hours on the ninth, tenth, or eleventh day of gestation. Nitrous oxide, oxygen, and halothane were delivered from an anesthesia machine (10 l/min total flow, Fluotec Mark 2 vaporizer) and introduced into the chamber via a metal inlet port. These gas flows were started 30 minutes before the hamsters were placed in the chamber. Excess gases escaped through an outlet port on the side opposite the inflow. A third opening, also opposite the inflow port, but closed with a metal three-way stopcock, was used to obtain gas samples from the chamber just before the hamsters were introduced and then one, two, and three hours later. These samples were analyzed for halothane, oxygen, and carbon dioxide concentrations. Gas chromatography

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confirmed a constant halothane concentration in the chamber of 0.6 per cent. Maintenance of chamber oxygen concentrations at 40 per cent as measured by an oxygen electrode indicated the presence of about 60 per cent nitrous oxide. Chamber carbon dioxide concentrations were always below the detection limits of the carbon dioxide electrode (1 per cent). The hamsters became immobile and appeared to be asleep 15–30 minutes after being placed in the chamber. When anesthesia was sufficient the hamsters were turned supine. After three hours they were removed from the chamber and were upright and mobile within 5 minutes.

The control hamsters were placed in a different but identical chamber from 9:00 A.M. to 12:00 noon on the ninth, tenth, or eleventh day of gestation. Air and oxygen from individual tanks were mixed before delivery into the chamber to provide an oxygen concentration of 40 per cent as measured with a Beckman oxygen analyzer. Food and water were withheld from both anesthetized and control animals during the treatment period. No more than three animals were placed in the chamber at the same time. Chamber temperatures remained between 22 and 24 C during all treatments.

The hamsters were sacrificed on day 15 of the 16-day pregnancy at about 10:00 A.M., when the fetuses were approximately 14.5 days old. The numbers of developed fetuses and fetal resorptions were counted and the sum considered the number of implantations. Fetuses were weighed with an analytical balance to the nearest milligram. Crown-rump length was determined to the nearest millimeter by use of calipers. Sex determination was made by observing and subjectively comparing the distance between the anal opening and the genital tubercle.⁸ To test the validity of these observations, the sex was also determined by dissection of 20 randomly selected fetuses and observation of the internal reproductive organs (*i.e.*, undescended testes in the male, uterus and ovaries in the female). These dissections confirmed the fetal sex as previously determined.

Data were analyzed using the Freeman-Tukey angular transformation⁶ to determine a mean value before calculating significance

levels for fetal resorption rate and sex ratio. No transformation was necessary for calculating significance levels of fetal weight and length. Significance levels were then determined in all cases using the Repeated *t*-test.

Results

The results are listed in table 1. Fetal lengths and weights were decreased and fetal resorptions were increased in groups exposed to the anesthetic mixture. However, these changes were dependent on the day of gestation when the hamsters were anesthetized.

Fetal resorptions, similar to spontaneous abortion in man, were significantly greater than those in controls only in animals anesthetized on day 11 ($P < 0.05$). Mean fetal weights were significantly decreased in animals anesthetized on days 10 and 11 ($P < 0.001$). Decreased crown-rump lengths were found in animals anesthetized on days 10 ($P < 0.001$) and 11 ($P < 0.02$). The ratios of female fetuses to total surviving fetuses were similar in the control and anesthetized hamsters.

Discussion

The selection of the hamster was influenced by the short gestation time (15 days and 17 hours)⁷ and the predictability of fetal development.⁸ For example, hamster fetal development on days 9, 10, and 11 of gestation should correspond closely to human fetal development occurring during weeks 4, 5, and 6, respectively.^{8,9} These weeks are considered a critical period of normal human embryogenesis during which drug-induced changes may result in developmental abnormalities.⁹ For these reasons it is felt prudent to defer elective anesthesia and operation during early pregnancy to after embryogenesis has been completed.¹⁰ This argument is further supported by findings that inhalation anesthetics are teratogenic in birds and/or mammals.¹ Furthermore, chronic exposure of operating room personnel to trace concentrations of inhalation anesthetics may be associated with increased incidences of spontaneous abortions and congenital anomalies.¹

This study was designed to duplicate an anesthetic drug combination that might be

TABLE I. Data from Pregnant Hamsters Anesthetized on Days 9, 10, and 11 Compared with Control Animals*

	Number of Pregnant Hamsters	Implantations	Resorptions	Fetal Weight (Mean \pm SE, mg)	Crown-Rump Length (Mean \pm SE, mm)	Sex Ratio (Females/Total Surviving Fetuses)
Day 9						
Nitrous oxide-halothane	8	107	4	1,686.8 \pm 22.9	23.7 \pm 0.2	.60
Control	9	110	9	1,737.1 \pm 27.7	23.9 \pm 0.2	.67
Day 10						
Nitrous oxide-halothane	9	102	30	1,489.0 \pm 22.1§	22.6 \pm 0.2§	.59
Control	9	103	11	1,916.5 \pm 22.7	24.9 \pm 0.2	.51
Day 11						
Nitrous oxide-halothane	9	109	16†	1,551.8 \pm 23.2§	22.7 \pm 0.2†	.65
Control	9	112	5	1,699.8 \pm 24.2	23.3 \pm 0.2	.63

* Implantations were the sum of developed fetuses plus fetal resorptions. The number of observations used for determination of fetal weight, length, and sex was implantations minus resorptions.

† $P < 0.05$.

‡ $P < 0.02$.

§ $P < 0.001$.

administered to a pregnant patient. The concentrations of nitrous oxide (60 per cent) and halothane (0.6 per cent) and duration of administration are similar to those which might be used during anesthesia and operation. Other investigators have studied nitrous oxide² and halothane³ alone, but not in combination. Likewise, the durations of administration have been prolonged. For example, Fink *et al.*² found that 45 to 50 per cent nitrous oxide was associated with increased fetal resorption and osseous abnormalities, but the shortest period of administration was 24 hours.¹¹ Basford and Fink³ found that osseous abnormalities were increased at certain stages of pregnancy when rats were exposed to 0.8 per cent halothane for 12 hours.

Fetal resorptions, which are similar to spontaneous abortions in man, were significantly greater than control only in animals anesthetized on day 11 ($P < 0.05$). At this time 16 of 109 implantations were resorptions, compared with 5 of 112 in the control group. Animals anesthetized on day 10 had 30 resorptions in 102 implantations, but this was not significantly different from the control value. This implies a large variation in the incidence of resorptions, and sporadic low or high incidences in either group may make inter-

pretation of the role of the anesthetics uncertain. Basford and Fink³ were unable clearly to demonstrate increased resorptions when halothane was administered to pregnant rats. However, the incidence of resorptions in those anesthetized on day 9 compared with control animals was moderately increased ($P < 0.05$). The same investigators found a diurnal variation in resorptions—more deaths occurred in those rats anesthetized between 9 A.M. and 9 P.M. than in those anesthetized between 9 P.M. and 9 A.M. All our hamsters were anesthetized during the day (9 A.M. to 12 noon). In the rat, nitrous oxide alone increased the resorption rate in proportion to the duration of exposure.² After 48 hours of exposure, 19 per cent of implantations were resorptions, and this increased to 57 per cent resorptions after 6 days of continuous exposure to nitrous oxide. Since our hamsters were anesthetized for only 3 hours, the impact of nitrous oxide and/or halothane may have been correspondingly reduced.

The decreased fetal weight and length of fetuses from hamsters anesthetized on days 10 and 11 suggested developmental retardation. However, supportive evidence, as reflected by osseous abnormalities, was not determined. In previous studies, nitrous oxide² and halo-

thane³ alone were associated with skeletal abnormalities.

More than half the surviving fetuses were judged to be females (table 1). At first glance, this suggested a specific male lethality resulting in a larger proportion of surviving fetuses' being female. However, the sex ratio (females/total surviving fetuses) was similar in the control group. Halothane alone was previously found not to alter the sex ratio, but male fetuses had a predominance of lumbar ribs.³ In contrast, nitrous oxide appeared to exert specific lethality on the male embryo.³

Other factors such as starvation or ventilation-related hypoxia and/or acidosis, body temperature changes, and maternal posture during anesthesia could conceivably result in abnormalities that would not be present in the control group. Starvation cannot be quantitated. However, anesthetized hamsters awakened rapidly and had free access to food and water. The inspired oxygen concentration of 40 per cent should have decreased any tendency for the development of arterial hypoxia during anesthesia. Arterial oxygen partial pressure determinations were not possible to document this assumption. Some carbon dioxide retention may have developed secondary to anesthesia-related respiratory depression, but high fresh gas flows prevented carbon dioxide accumulation in the chamber. Temperatures in the chambers were similar for both groups. Hamsters were placed supine when anesthesia had been established in an attempt to decrease the possibility of increased pressure on the uterus.

Compared with control hamsters, fetal changes occurred in hamsters exposed for three hours to anesthetic concentrations of nitrous oxide plus halothane. Although animal data cannot be readily extrapolated to humans, the presence of any change in the anes-

thetized hamsters reinforces the clinical impression that drugs, including inhalation anesthetics, should be avoided if possible during early human pregnancy.

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