The Teratogenicity of Halothane in the Rat

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Rats were exposed to 0.8 per cent halothane for 12-hour periods at different stages of pregnancy. Fetuses near term were suitably prepared and the skeletons examined. Lumbar ribs and separation of normally single ossification centers into paired lateral centers in the lower thoracic vertebral bodies were common. Incidences of these anomalies were compared with those of a control series and found significantly higher following exposure to halothane on day 8 or day 9½ (or day 10 for ribs only). Diurnal variation in the incidence of resorptions following halothane was noted, more fetal deaths occurring after exposure during the day than during the night. The anomalies found are similar to those seen following exposure to nitrous oxide.

GROWING AWARENESS that many therapeutic agents in common clinical use have not been completely evaluated for teratogenicity has stimulated considerable interest in determining this potential for general anesthetics. Most of the work to date has been done on incubating chicks. Increased incidence of fetal death, development of anomalies, and retarded growth have been reported by various authors following exposure of eggs to nitrous oxide, ether, cyclopropane, halothane (Fluothane), methoxyflurane (Penthrane), fluroxene, or tetraflurobromethane.[1-7]

The administration of anesthetics to mammalian embryos increases experimental complexity considerably by adding diffusion barriers and introducing the possibility of effects from maternal respiratory or cardiovascular depression, but the desirability of evaluating teratogenicity in an animal related more closely to man than the chick is obvious. Fink, Shepard and Blandau,[8] using rats, reported retarded fetal growth as well as increased rates of death and malformation following exposure to nitrous oxide for periods of 48 hours or more. A later report by this group[9] confirmed the presence of teratogenic effects following 24-hour exposure.

Halothane is of particular interest as a potential teratogen, not only because of its wide clinical usage, but because there are several reports indicating interference with growth of rapidly proliferating cells under other circumstances. Bruce and Koepke[10] reported depression of granulopoiesis in rats exposed to halothane for several days. Fink and Kenny[11,12] described inhibition of growth in several strains of mammalian cells in culture, the degree of depression being closely related to the concentration of halothane administered. It seems reasonable to suppose that sufficient interference with cell proliferation in the developing embryo might, at a critical time, result in abnormal development. The objective of this study was to investigate the susceptibility of the rat embryo to damage by an anesthetic concentration of halothane and to determine the stage of development during which the embryo is most vulnerable.

Materials and Methods

Female Sprague–Dawley rats in estrus were placed with males overnight and adjudged pregnant the following morning if copulation plugs or vaginal sperm were found. The day on which pregnancy was diagnosed was counted as day 0, starting at 9 a.m.

The pregnant rats were divided into nine experimental and nine control groups. Management of all groups was the same except for a single treatment period. Experimental

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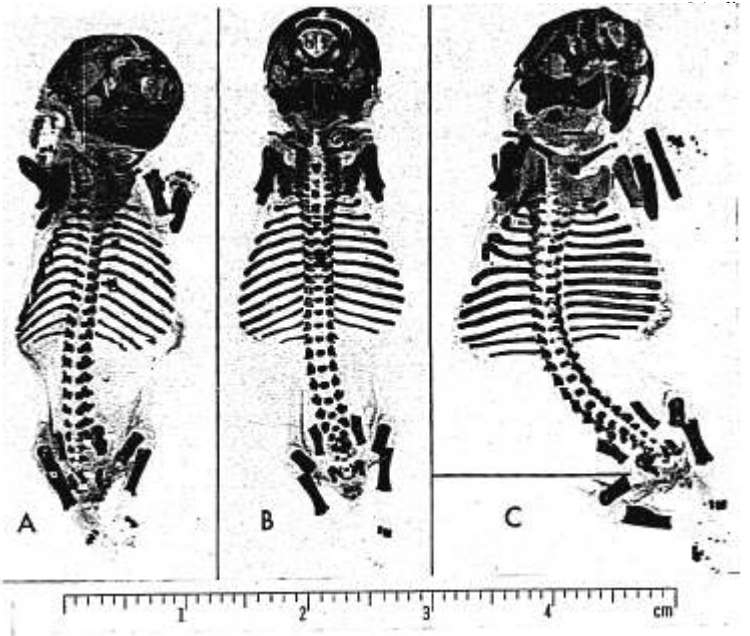

FIG. 1. Fetuses prepared for examination of the skeleton. Control at left is normal. Center shows lumbar rib and rib rudiment. Fetus at right shows, in addition to lumbar rib rudiments, completely separated ossification centers in the bodies of T4, T6, T9, and T12, with partial separation at T7 and T11. Uniform magnification.

groups received 0.8 per cent halothane ‡ in 25 per cent oxygen–nitrogen for 12 hours, and were allowed free access to food and water. This gas mixture caused light sleep within an hour with the animals being progressively more difficult to rouse. By 6–10 hours, they did not respond to moving the box, but some responded to painful stimuli throughout the 12 hours. They ate and drank little during treatment and some continued to lose weight for about 24 hours after return to their cages. Control groups breathed air and were deprived of both food and water during the treatment period. The control animals lost more weight during the test period but regained it more rapidly, and we believe that the starvation stress was roughly comparable in the two regimens.

Groups of animals were treated on day 6, 7, 8, 9, or 10 (9 a.m. to 9 p.m.) or 6½, 7½, 8½, or 9½ (9 p.m. to 9 a.m.). Throughout the study comparisons were made between groups exposed to the two different regimens at comparable times. In addition, the pooled data ("totals") from the nine groups exposed to the experimental regimen were compared with those from the nine control groups.

Treatment was accomplished in sealed glass-top 10-liter metal boxes with wire mesh floors over layers of soda lime. Gases flowed through the boxes at a rate of two liters per minute from previously calibrated flowmeters. Not

‡ Fluothane, kindly supplied by Ayerst Laboratories.

TABLE 1. Incidence of Vertebral Anomalies Following Experimental and Control Regimens

Day of Exposure	Treatment				Statistical Significance of Differences	
	Halothane		Control			
	Number of Fetuses	Per Cent with Anomaly	Number of Fetuses	Per Cent with Anomaly	X^2	P
6	95 (9)*	33.7	97 (9)	39.2	0.62	0.43
6½	96 (8)	33.3	108(10)	45.4	3.41	0.07
7	98 (9)	40.8	100 (9)	36.0	0.12	0.73
7½	113 (8)	38.0	114 (9)	31.6	2.03	0.15
8	104 (9)	44.2	102 (9)	29.4	4.74	0.03†
8½	99 (8)	36.4	107 (9)	35.5	0.13	0.72
9	101 (9)	53.5	98 (9)	38.8	2.18	0.14
9½	107 (9)	54.2	99 (8)	21.2	12.45	<0.001‡
10	98 (8)	30.6	112 (9)	42.9	3.34	0.07
TOTALS	911(77)	40.7	937(81)	35.6	5.05	0.02†

* Number of litters in parentheses.
† Significant.
‡ Highly significant.

more than three rats were treated in one box at any given time. The concentration of carbon dioxide in the outflow gas, measured periodically by infrared analyzer, did not exceed 0.2 per cent. The halothane concentration was calculated from the vapor pressure and confirmed by measurement of the amount of liquid halothane vaporized.

Arterial gas tensions were measured in a separate series of rats to determine the relative effects of the gas mixtures administered to the experimental and control groups. Blood for these measurements was obtained from femoral artery cutdowns done under local anesthesia.

On day 20 the rats were sacrificed. The number of fetuses and resorptions and their locations in the uterus were noted. Fetuses were examined for weight, length, sex, and gross appearance, and labeled with silver McKenzie clips. They were then fixed in acetone–alcohol and prepared with potassium hydroxide, alizarin red S, and glycerine for examination of the skeleton by our modification of the

TABLE 2. Incidence of Lumbar Ribs Following Experimental and Control Regimens

Day of Exposure	Treatment				Statistical Significance of Differences	
	Halothane		Control			
	Number of Fetuses	Per Cent with Anomaly	Number of Fetuses	Per Cent with Anomaly	X^2	P
6	86	27.4	96	18.8	1.88	0.17
6½	78	17.9	108	37.0	8.01	0.002†
7	98	24.5	100	19.0	0.95	0.33
7½	113	10.6	114	18.4	1.58	0.21
8	104	26.9	102	5.9	16.88	<0.001‡
8½	99	22.2	107	21.5	0.02	>0.75
9	101	25.7	98	18.4	2.25	0.14
9½	107	65.4	99	32.3	13.69	<0.001‡
10	98	50.0	112	19.6	21.53	<0.001‡
TOTALS	884	30.7	936	21.2	17.81	<0.001‡

† Significant.
‡ Highly significant.

TABLE 3. Incidence of Resorptions Following Experimental and Control Regimens

Day of Exposure	Treatment				Statistical Significance of Differences	
	Halothane		Control			
	Implantations	Resorptions	Implantations	Resorptions	X^2	P
6	98	2.04	111	12.61	7.07	0.01†
6½	100	2.00	124	12.9	10.24	0.002†
7	114	14.04	105	4.76	2.92	0.08
7½	114	1.75	118	3.39	0.55	0.46
8	111	6.30	106	3.77	1.07	0.31
8½	102	2.94	110	2.72	0.14	0.71
9	110	8.18	99	1.01	8.07	0.01†
9½	108	0.93	102	2.94	0.92	0.33
10	106	7.55	116	3.40	2.02	0.15
TOTALS	963	5.19	992	5.44	0.06	0.8

† Significant.

technique described by Lippman.[13] Approximately 100 fetuses were studied in each of the 18 groups.

Results

In order to avoid bias, all fetuses were examined without the examiner's knowing to which regimen they had been subjected. Two anomalies of the skeleton were common: (1) separate or partly fused lateral ossification centers, rather than the normal single centrum in the lower thoracic and upper lumbar vertebral bodies; and (2) lumbar ribs or rib rudiments (fig. 1). Both of these malformations occur occasionally in normal rats as well as in the starved controls.

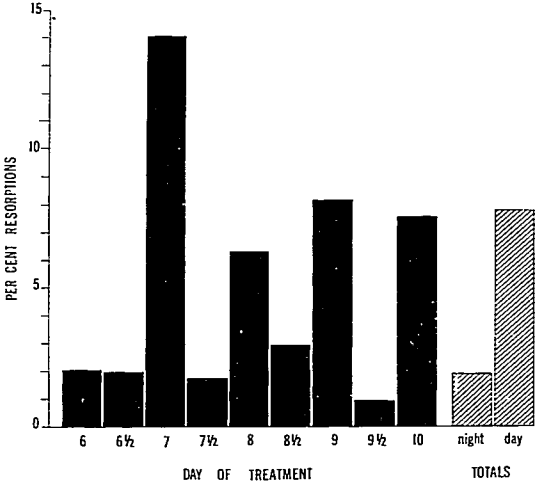

FIG. 2. Incidences of resorptions following exposure to halothane at various times.

The incidences of vertebral anomalies following experimental and control measurements at the various times of exposure are indicated in table 1. The significance of differences between regimens was calculated using the Chi-square test, with Yates' correction where applicable.

The incidence of vertebral anomalies following exposure to halothane was greater than that following the control regimen in all treatment periods from day 7 through day 9½, the difference being significant on days 8 and 9½ and in the "totals." The peak incidence of vertebral malformations followed exposure on day 9½, the difference between halothane and control here being highly significant ($P < 0.001$).

The incidences of lumbar ribs following the two regimens are shown in table 2. Exposure to halothane in all periods from day 8 through day 10 resulted in more anomalous ribs than in the controls. The maximum incidence of this abnormality also followed exposure on day 9½, and the differences between halothane and control were highly significant on days 8, 9½, 10, and in the "totals." The control regimen on day 6½ produced a significantly higher incidence of rib anomalies than did halothane.

Table 3 indicates the incidence of resorptions. Although moderate significance ($P < 0.05$) was noted in the differences between experimental and control groups on days 6, 6½ and 9, the incidence of resorptions was erratic. The difference between experimental and control groups was not significant in the "totals," and the role of halothane uncertain.

A graph of the data relating to incidence of resorptions (fig. 2) shows distinct diurnal variation, more resorptions following exposure to halothane during the day than during the night. This difference is significant at the $P < 0.001$ level. Vertebral and rib anomalies showed a similar trend but at much lower levels of significance. Diurnal variations are known to occur in many physiologic functions, and a resulting variation in susceptibility to halothane is not surprising. This correlates well with Matthews' recent work[14] indicating that mice are more susceptible to the toxic effects of halothane during their normal period of sleep.

The weights of the fetuses subjected to halothane did not differ significantly from the weights of controls.

The halothane-treated group showed the normal preponderance of males, 54 per cent, while the control group showed a decreased proportion of males, 48 per cent. No difference could be demonstrated between males and females in the incidence of vertebral anomalies, but males appeared to be more susceptible to the development of lumbar ribs ($P = 0.05$).

Arterial oxygen and carbon dioxide tensions and *p*H were measured in pregnant (days 7–9) and nonpregnant rats to determine the effects of halothane on these factors (table 4). Each animal was its own control, and determinations during administration of halothane were made after at least ten hours of exposure to 0.8 per cent.

The data were analyzed for any indication that size of litter or position of the fetus in the uterus might affect fetal weight, resorptions, or anomalies. No correlations among any of these parameters could be established.

TABLE 4. Arterial Blood Gases in Rats Exposed to Gas Mixtures Used for Experimental and Control Regimens and to Halothane in Air without Supplemental Oxygen

	Inspired Gas	P_{O_2}	P_{CO_2}	pH
Nonpregnant 8 rats	Air	95.0 ± 6.6*	32.1 ± 2.6	7.47
	Halothane in air	91.5 ± 13.0	41.1 ± 7.7	7.45
	Halothane in 25 per cent O_2	104.4 ± 16.4	41.4 ± 7.4	7.44
Pregnant 4 rats	Air	98.0 ± 5.5	30.5 ± 2.5	7.43
	Halothane in air	90.8 ± 8.8	50.7 ± 5.3	7.34
	Halothane in 25 per cent O_2	99.0 ± 8.9	46.2 ± 9.5	7.34

* Mean ± standard deviation.

Discussion

In determining the procedures for this study, the concentration of halothane and the length of exposure used were chosen after preliminary experiments showed this to be the longest time for which rats could be exposed to a light anesthetic concentration without risk of undue respiratory or cardiovascular depression.

It is surmised that the low CO_2 tensions seen in both pregnant and nonpregnant animals breathing air probably represent acute hyperventilation in awake restrained rats; the relatively high *p*H's tend to support this. The fact that P_{CO_2} and *p*H in animals breathing air were both lower in the pregnant than in nonpregnant rats may indicate that rats have the same phenomenon of chronic hyperventilation in response to the hormonal changes of pregnancy that is known to occur in humans.[15] Because of the decrease in P_{O_2} following administration of halothane in air, 25 per cent oxygen was used in the study of teratogenicity to prevent hypoxia. These measurements lead us to believe that the pregnant animals in this study were normally oxygenated although P_{CO_2}'s probably were mildly elevated.

The functional as opposed to statistical significance of the malformations found is uncertain. Both vertebral and rib anomalies represent abnormal development rather than simple retardation of normal development, but may not interfere with normal function of the animal. The method of preparation prevented examination of tissues other than the skeleton.

The question arose whether the results would differ if the unit for analysis was the individual fetus or the litter. Therefore, analysis of the percentage of abnormal fetuses in litters subjected to the different regimens was also done, using the unpaired Student's *t* test. Levels of significance were somewhat lower, but the general findings were similar.

It was also noted that incidences of both rib and vertebral anomalies in the controls were quite variable, and the possibility that significant differences might be due to sporadic low incidences in the controls rather than high incidences in the experimental groups was raised. Accordingly, the incidences of anomalies found significantly greater than controls by the above analysis were compared with incidences in the control "totals." This eliminated significant differences on days 6½ and 8, but the differences on days 9½ and, for ribs only, 10 remained highly significant ($P < 0.001$).

The fetal abnormalities seen following halothane in rats are strikingly similar both in kind and in frequency to those seen following nitrous oxide.[9] This suggests that teratogenicity may be a general property of anesthetics rather than a specific pharmacologic effect of the individual drug.

Maximal susceptibility to rib and vertebral malformation occurred in this study on days 8 through 10. During this time, the rat embryo progresses through the primitive streak and neurula stages and, by day 10½, has formed the somites from the cranium through the lower thoracic region.[16] This corresponds approximately to the fourth week after conception in the human.

One aspect of the species differences between rats and man that is difficult to evaluate is the relative importance of a given length of time in the two species. The lifespan of man is many times longer than the rat's, and the length of gestation is about 13 times as great, but the rat is far less mature at birth. The time required to reach the thoracic somite stage is less than three times longer for humans, and the length of the cell cycle, from one division to the next, is essentially the same for both species. Which, if any, of these comparisons is pertinent to teratologic studies is unknown.

The important question, whether anesthetics are teratogenic in humans, cannot be answered at present. Experimentation is obviously impossible, and there is a paucity of even retrospective clinical studies. Shnider's recent summary[17] suggests some of the difficulties encountered. While studies in humans must ultimately be done, perhaps indication of what might be expected could be gained sooner from studies of other primates.

However, the finding that halothane and nitrous oxide are teratogenic in rats is cause for concern. Rats are mammals; the effects on bone marrow from nitrous oxide are similar to the human's; the concentrations of both nitrous oxide and halothane required to produce

various depths of anesthesia appear to be essentially the same as required by humans. It would appear that the general caution to avoid administration of any drugs to women in early pregnancy unless specifically necessary might well be extended to include the general anesthetics.

Conclusions

Halothane appears to be teratogenic in rats, the degree of damage varying directly with concentration and duration of exposure during a susceptible period. While direct extrapolation of these data to humans is unjustified, this study suggests the need for further investigation of the teratogenic potential of general anesthetic agents.

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Surgery

ACCIDENTAL ARTERIAL INJECTION Accidental intra-arterial injection of meperidine and promethazine occurred in a patient in labor. The site of injection was the antecubital fossa. Pain, blanching, and cyanosis of the hand quickly followed. Treatment consisted of heparin and corticosteroids, resulting in complete recovery. The injury is probably a chemical endarteritis caused by a high concentration of drug delivered to the peripheral vessels. On examination, the patient was found to have a superficial, malpositioned radial artery. (*Webb, G., and Lampert, N.: Accidental Arterial Injection, Amer. J. Obstet. Gynec. 101: 365 (June) 1968.*)