INHALED ANAESTHETICS HAVE NO EFFECT ON FERTILITY IN DROSOPHILA MELANOGASTER

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Epidemiological studies have led to the suggestion that an increased frequency of adverse reproductive effects may follow occupational exposure to waste anaesthetic gases. Spontaneous abortion among women directly exposed to anaesthetics and among wives of men exposed, and congenital anomalies among their offspring have been the focus of most studies (NIOSH, 1977; Ferstandig, 1978; Vessey, 1978; Baden and Rice, 1981). In addition, Knill-Jones and colleagues (1972) reported that the frequency of infertility among female operating suite personnel was twice as high as a comparable control group. Greater frequency of involuntary infertility among women in anaesthetic practice has also been reported by Tomlin (1979). In animals, the potential abortifacient and teratogenic effects of anaesthetic agents have been extensively studied. However, the effects of anaesthetics on fertility have not been systematically studied, even though there is limited evidence to suggest that these agents disrupt germ cells under certain conditions. Thus, the present study was undertaken to determine the effects of several anaesthetics on fertility under similar experimental conditions. The fruit fly, Drosophila melanogaster, a species widely used for genetic testing, was chosen as the test model.

MATERIALS AND METHODS

Two-day-old flies of a wild-type strain (Oregon R) of Drosophila melanogaster were used. All flies were reared in vials containing instant medium (Carolina Biological Supply, Burlington, NC). The cultures were maintained at a temperature of 25 ± 1 °C and a relative humidity of 50 ± 5%. The following anaesthetics were tested: enflurane, isoflurane, halothane, fluroxene and nitrous oxide. It was first necessary to establish the LD₅₀ values for each agent so that appropriate concentrations could be chosen for the fertility tests. For enflurane, isoflurane and halothane, groups of 100 males and 100 females were exposed to 0, 0.5, 1, 2, 4, 6, 7, 8, or 16%. For fluroxene, two additional concentrations, 20 and 24% were used. For nitrous oxide, the concentrations were 0, 20, 40, 50, 60, 80 and 100%.

All exposures were for 1 h at 25 °C. During exposure, the concentration of oxygen was maintained between 19 and 23%, except for the 100% nitrous oxide group, for which it was 0%. The concentration of oxygen was monitored by an IL 402 Oxygen Monitor (Instrumental Laboratory, Lexington, Ma). Gas chromatographic measurements or infra-red gas analyses of anaesthetic concentrations were obtained at the beginning and end of exposure and showed that the concentration of the anaesthetics did not de-
increase by more than 5%. Following treatment, flies were returned to clean vials with fresh medium, and were counted 24 h later to determine the one day LD_{50} values using the probit analysis procedure. Fertility tests were performed at all concentrations up to and including 7% for volatile agents and 100% for nitrous oxide. No fertility tests were undertaken above 7% for the volatile anaesthetics because concentrations greater than this percentage were lethal to 50% or more of the fly population.

Immediately after counting, five males were selected at random from each treatment group. Each male was mated for 3 days to two untreated virgin females in individual vials with fresh medium. Males were then removed and immediately individually mated with two fresh females to produce the second brood. The mating procedure was repeated once more to produce the third brood. In addition, 10 treated virgin females were selected at random from each treatment group and allowed to mate in pairs with one untreated male; the same pattern of brooding was followed as for the treated males. The successive broods represent cells treated at different stages of spermatogenesis or at increasing times after oogenesis. The numbers of live male and female progeny produced for each brood were counted. The data were analysed using probit analysis and the Chi-square (\(\chi^2\)) test. \(P < 0.05\) was considered statistically significant.

RESULTS

From the percentage of flies dying at each dose over a 24-h period, the 1-day LD_{50} was calculated for each anaesthetic using probit analysis (table I).

### Table I. LD_{50} (% v/v) values 24 h after an acute exposure of 1 h.

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>LD_{50} values</th>
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<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>Enflurane</td>
<td>8.96</td>
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<tr>
<td>Isoflurane</td>
<td>7.80</td>
</tr>
<tr>
<td>Halothane</td>
<td>7.29</td>
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<tr>
<td>Fluroxene</td>
<td>20.37*</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>**</td>
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</tbody>
</table>

* Values significantly greater than those observed with other anaesthetics. ** No deaths observed.
Statistically, there were no differences in LD_{so} values for enfurane, iso-Surane, and halothane with the values ranging between 7.29 and 8.96%. Values for fluroxene, however, were significantly greater than for the other anaesthetics (P < 0.01); an average dose of 20.5% of fluroxene was required for a one-day LD_{so}. Ninety-five percent or more of the flies succumbed within 24 h of treatment at a concentration of 16% for enfurane, iso-Surane and halothane. Flies treated with fluroxene were more resistant: a concentration of 24% was required for 100% lethality. Interestingly, all flies exposed to 100% nitrous oxide survived, indicating that this species is extremely resistant to hypoxia.

There was no effect on fertility which could be related to treatment. That is, there were no significant differences of brood or total numbers of male and female offspring among the various treatment groups when data were analysed by χ^2 (table II).

**DISCUSSION**

*Drosophila melanogaster* was selected for this study because it is easily and economically reared and has a short generation time of about 10 days at 25°C. Furthermore, next to man, its genetics and reproduction are best known. Thus, *Drosophila* has been used widely to study chemically-induced mutagenic and adverse reproductive effects. Infertility caused by drugs is one of the effects observed in this species (Dybas, Tyl and Geer, 1981; Nikki and Okada, 1981; Ranganath and Krishnamurthy, 1981; Brink, 1982). For example, Brink (1982) reported an increase in sterility when male and female adults as well as larvae of *Drosophila* were fed the pyrolozidine alkaloid, heliotrine. Moreover, an increased frequency of congenital anomalies in the abdomen of the fruit fly was noted. Such studies have led to the acceptance of *Drosophila* as a model for testing the general effects of drugs on fertility. Furthermore, use of the brooding technique in *Drosophila* enables determination of the specific stage of germ-cell development which is affected by a chemical.

Epidemiological studies have found an increased frequency of involuntary infertility in operating room personnel (Knill-Jones et al., 1972; Tomlin, 1979). However, many factors other than exposure to waste anaesthetic gases may have accounted for these findings. Several investigators have examined the effects of anaesthetics on fertility of rodents (Pope et al., 1978; Wharton et al., 1979; Wharton, Mazze and Wilson, 1981; Mazze et al., 1982) but results have been uniformly negative.

In addition to fertility studies, several attempts have been made to investigate the direct effects of anaesthetics on male and female germ cells in rodents. Atrophy of the seminiferous tubules, decreased sperm count and decreased testicular weight in rats exposed to nitrous oxide continuously or acutely have been reported (Kripke et al., 1976). Adverse effects were observed as early as 48 h after treatment was initiated, but were rapidly reversible when exposures ceased. Coate, Kapp and Lewis (1979) subjected rats to halothane 1 p.p.m. plus nitrous oxide 50 p.p.m., or to halothane 10 p.p.m. plus nitrous oxide 500 p.p.m. After 48 weeks of exposure, the frequency of chromosomal aberrations in spermatogonial cells from rats exposed to a mixture of nitrous oxide – halothane was significantly greater than from rats subjected only to air. However, the types of aberration noted probably were present too infrequently to cause decreased fertility and were not heritable. Land, Owen and Linde (1981) examined the sperm of mice exposed to various anaesthetics for 4 h per day, 5 days per week for 14 weeks. It was found that the percentages of abnormal sperm following exposure to 0.08% chloroform, 0.2% trichloroethylene or 1.0% enfurane were 3.5, 2.4, and 2.0%, respectively, which were slightly but significantly above the control value (1.4%). In contrast, Baden and colleagues (1980) observed a normal rate of abnormal sperm in Swiss/ICR mice exposed to 0.3% enfurane, 4 h per day, 5 days per week for 52 weeks. No increase in the rate of chromosomal aberrations in spermatogonial cells was noted. Overall, these studies indicate that anaesthetics have mild toxic effects on male germ cells which are, however, unlikely to be associated with infertility.

Most of these animal studies have been performed at different times, in different laboratories, under a variety of experimental conditions. Thus, it is difficult to obtain a consensus of absolute or relative risk among the agents. Our study is an attempt to examine several anaesthetics agents at one time under standard conditions in a well-validated model for infertility. Although not excluding the possibility that anaesthetics decrease fertility in man, our negative results in the fruit fly, together with those of others in rodents, must be regarded as encouraging for both operating room personnel and patients alike.
ACKNOWLEDGEMENT

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REFERENCES


