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Sister Chromatid Exchanges Induced by Inhaled Anesthetics

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There is sufficient evidence that anesthetics may cause cancer to justify a test of their carcinogenic potential. Baden *et al.*, using the Ames test, a rapid and inexpensive genetic indicator of carcinogenicity, have shown that among currently used anesthetics fluroxene alone caused bacterial mutations. The authors used the sister chromatid exchange (SCE) technique, another rapid assay of mutagenic–carcinogenic potential. The frequency of sister chromatid exchanges in Chinese hamster ovary cells increases when the cell cultures are exposed to mutagen-carcinogens, particulary in the presence of a metabolic activating system. With this test system a one-hour exposure to 1 MAC nitrous oxide, diethyl ether, trichloroethylene, halothane, enflurane, isoflurane, methoxyflurane, or chloroform did not increase SCE values. Divinyl ether, fluroxene and ethyl vinyl ether increased SCE values in the same circumstances. Results of this study of mammalian cells suggest that no currently used anesthetic is a mutagen–carcinogen. The results also suggest that anesthetics containing a vinyl moiety may be mutagen–carcinogens. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: chloroform; diethyl ether; divinyl ether; enflurane; ethyl vinyl ether; fluroxene; halothane; isoflurane; methoxyflurane; trichloroethylene. Cancer. Toxicity: mutagenesis.)

THE EVIDENCE that inhaled anesthetics may cause cancer[1–3] is sufficient to justify a test of the carcinogenic potential of these drugs. The standard bioassay for chemical carcinogenesis requires lifetime exposure of large numbers of animals, usually mice or rats. However, this approach is costly and time-consuming. Alternative approaches are simple rapid screening tests that use genetic damage as an indicator of carcinogenicity. One such test is the Ames test,[4] which measures reverse mutations in *Salmonella typhimurium* after exposure to a potential carcinogen. Using this test with each of the currently and several previously used inhaled anesthetics, Baden found that fluoroxene, but not other anesthetics, caused mutations.[5]

Another rapid *in-vitro* assay of genetic damage measures the exchange of chromosomal DNA that occurs during replication. The test reveals the exchange of chromosomal material between sister chromatids during cell division. Exposure to known mutagen–carcinogens during cell division increases the frequency of sister chromatid exchanges.[6] These increases can follow treatment with doses of a carcinogen that are insufficient to cause gross morphologic chromosomal changes. The exchanges are tested in Chinese hamster ovary cells growing in culture. As with the Ames system, a rat liver microsome metabolizing system may be included.[7,8] The sister chromatid exchange assay thus comprises a mammalian test of the mutagenicity of both the parent compound and its metabolites.

In the present study, we used the sister chromatid exchange assay to examine the carcinogenic potentials of ten inhaled anesthetics.

Materials and Methods

We added anesthetic drugs in carbon dioxide, 5 per cent, plus oxygen, 19 per cent, with the balance being nitrogen, to Chinese hamster ovary cells (CHO) growing exponentially. The anesthetic drug was delivered from calibrated cylinders into the culture flasks for 2 min. The flasks were then quickly capped and incubated at 37 C for one hour. A control value was obtained for each experiment by exposing cultures to carbon dioxide, 5 per cent, in air rather than the anesthetic agent for the one-hour treatment period. After the one-hour treatment, samples of the atmosphere above the culture medium were subjected to gas chromatographic analysis. The anesthetic was allowed to escape from the flask and the culture medium was replaced as indicated below.

We then used the methods of Perry and Wolff[9] to measure sister chromatid exchanges. Following the exposure to the anesthetic drug, the culture medium was changed to include bromdeoxyuridine (BrdUrd, 10 μM final concentration) in McCoy's 5A medium containing fetal calf serum, 15 per cent. After 24 hours, Colcemid (2×10^{-7} M) was added for two hours to arrest the mitotic cells at metaphase, and the cells were harvested by shaking. The cells were treated with 0.075 M KCl for 3–4 min to spread the chromosomes and then were fixed in methanol/acetic acid (3:1 v/v). Drops of cells in fixative were placed on slides and allowed to dry, stained in Hoechst 33258 (50 μg/ml in M/15 Sorenson's buffer, *p*H 6.8) for 15 min, and rinsed in distilled water. Using a few drops of the same buffer, a coverslip was mounted on each slide before it was exposed to light from a 100-w high-pressure mercury burner at 1.5 cm for 2–3 min.

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The coverslips were removed and the slides stained in 3 per cent Giemsa (Gurr's R66 in M/15 Sorenson's buffer, *p*H 6.8).

During the 24-hour period of growth in BrdUrd the cells undergo two rounds of DNA replication. Bromdeoxyuridine, a thymine analog, is incorporated into the newly made chromosomes. The fluorescence-plus-Giemsa (FPG) staining procedure renders half-substituted chromatids darkly stained and the fully substituted chromatids faintly stained. An exchange is counted for each change in darkness on the harlequin-patterned chromosomes (figs. 1 and 2).

To test the effects of anesthetic metabolites we used the methods described by Takehisa and Wolff.[10] Briefly, during the one-hour treatment with test gas, the medium included a S-9 rat liver extracted, 10 per cent (by volume), prepared from Aroclor 1254-treated male rats as directed by Ames *et al.*,[4] and an NADPH generating system. The culture medium was replaced with McCoy's 5A medium supplemented with fetal calf serum, 2.5 per cent, immediately before the one-hour treatment period. After the treatment period the cells were washed twice with fresh McCoy's 5A medium supplemented with fetal calf serum, 15 per cent, and the fresh complete medium with 10 μM BrdUrd was used for the final 24-hour growth period before harvest and fixation.

A single observer counted sister chromatid exchanges in 100 cells from each anesthetic treatment.

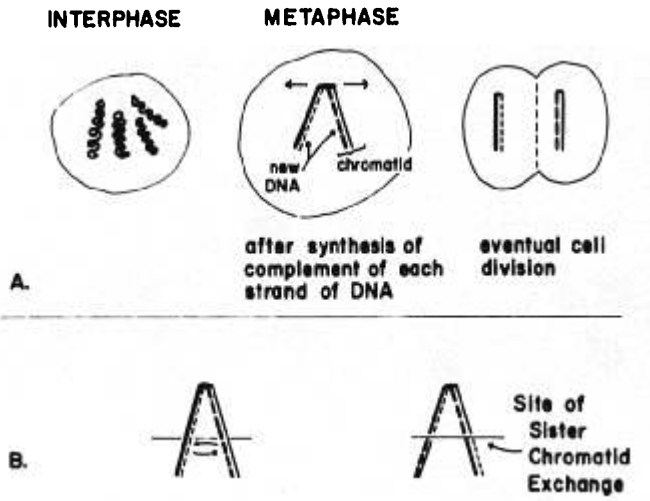

FIG. 1. *A*, in the routine course of mitosis, interphase chromosomes condense at metaphase, the old (*solid line*) strands of DNA separate, and new (*dashed lines*) complementary DNA in synthesized. Eventually the sister chromatids separate as cell division is complete.

B, when an exchange takes place between sister chromatids, old DNA becomes attached to new DNA, shown here as a DNA strand consisting of a solid-line section and a dashed-line section

An exchange was counted for each change from light to dark or from dark to light (fig. 3). The number of sister chromatid exchanges/chromosome was calculated for each cell. The numbers for 100 cells for each treatment were compared with the results for other treatments for that set by an analysis of variance.

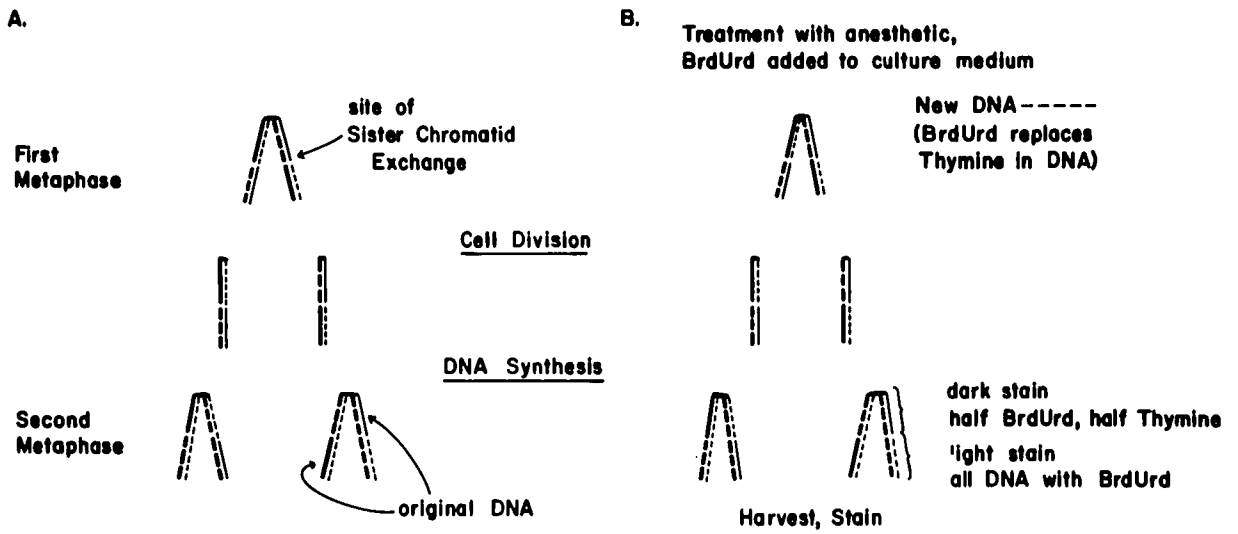

FIG. 2. *A*, in studying a sister chromatid exchange (*top*), one follows the chromosomes through the cell division after the exchange (*middle*) and through a second replication of DNA (*bottom*). At the second metaphase there is a single strand of the original DNA in parts of both chromatids. However, the original DNA alternates between the chromatids.

B, when bromdeoxyuridine (BrdUrd) is added to the culture medium, it replaces thymine in newly made DNA (*dashed line*). To visualize chromatid exchanges using the fluorescence-plus-Giemsa (FPG) technique, BrdUrd is added for two cycles of DNA replication. When BrdUrd has replaced thymine in both strands of the DNA, that section of chromosome stains more faintly than when BrdUrd is in one strand and thymine in the other. The solid-line sections of DNA are the only ones still containing thymine. These dark-staining areas alternate between chromatids, resulting in a harlequin appearance when there has been a sister chromatid exchange.

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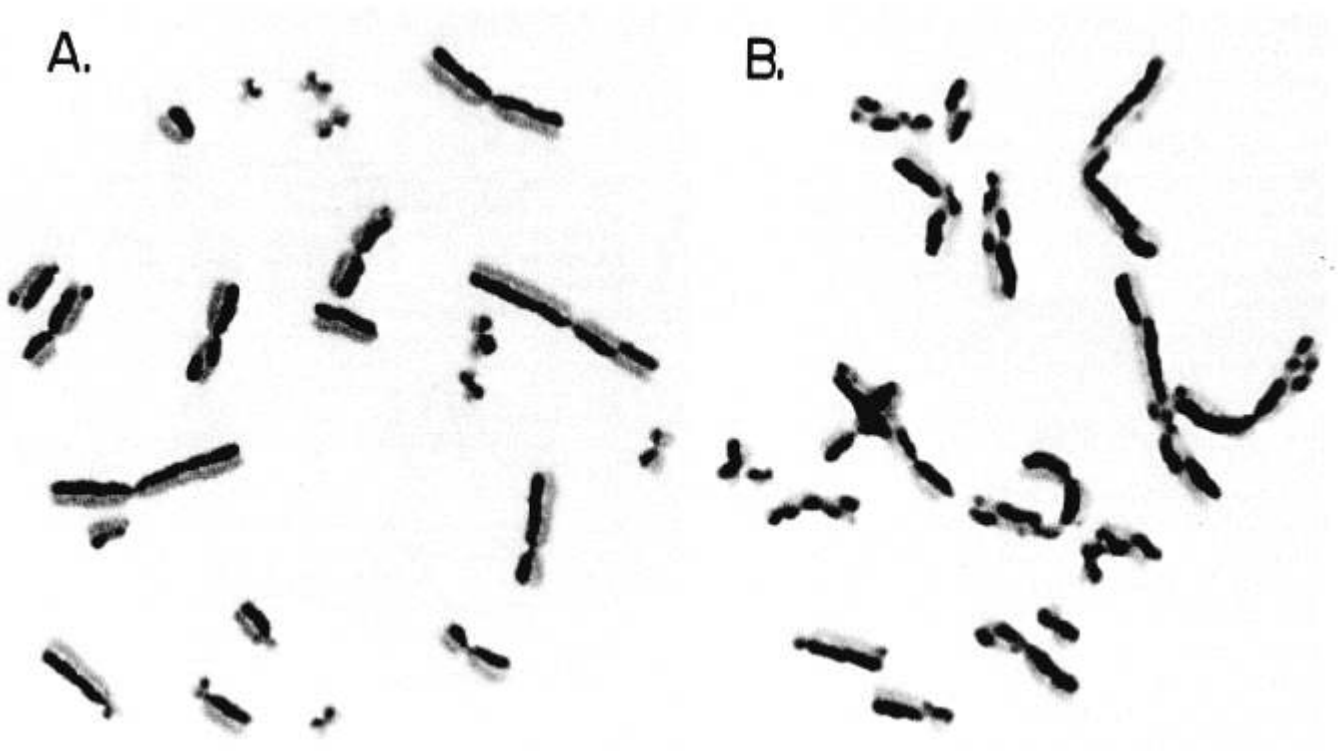

FIG. 3. Harlequin-patterned Chinese hamster ovary chromosomes. *A*, control; *B*, treated with sister chromatid exchange inducers.

The Student range test was used to determine the level of significance. The observer was blind to the treatment of the 100-cell groups.

Three sets of experiments were conducted. CHO cells were exposed in the presence of liver microsomes to nitrous oxide, diethyl ether, trichloroethylene, halothane, enflurane, chloroform, divinyl ether, fluroxene, and ethyl vinyl ether. Each anesthetic was administered at approximately 1.0 human MAC either determined or estimated, except that for nitrous oxide 0.64 MAC was used.[11] Additional CHO cells were exposed in the presence of liver microsomes to isoflurane, 1 and 10 MAC, and methoxyflurane, 1 and 6 MAC. Finally, additional CHO cells were exposed to 1 MAC each isoflurane, halothane, enflurane, fluroxene, methoxyflurane, or 0.64 MAC nitrous oxide for 24 hours without microsomal enzymes. Carbon dioxide, 5 per cent in air, was the control. To accomplish this the original culture medium was changed to include 10 μM BrdUrd prior to the exposure to the anesthetic. The flasks were quickly capped and sampled the next day just before Colcemid was added.

Results

After one hour of exposure, the anesthetic concentrations in the flasks equalled 70–98 per cent of those delivered from the tanks, except that in the trichloroethylene flask the concentration was 59 per cent of that delivered (table 1). After 24 hours of exposure, 80–92 per cent of the administered concentrations remained in the flasks, with the exception of the methoxyflurane flask, in which 43 per cent of the original samples remained.

Divinyl ether, fluroxene and ethyl vinyl ether, all vinyl-containing compounds, increased sister chromatid exchanges to significantly above the levels of the controls. Ethyl vinyl ether induced more exchanges than fluroxene, which, in turn, produced more exchanges than divinyl ether. No other agent in this group increased the exchanges to above control levels. Neither isoflurane at 1 or 10 MAC nor methoxyflurane at 1.0 or 6 MAC increased sister chromatid exchanges. There was no difference among the sister chromatid exchanges seen after 24 hours of exposure to isoflurane, enflurane, halothane, fluroxene, methoxyflurane, and nitrous oxide, compared with each other or with the control levels of exchanges.

Discussion

None of the anesthetics previously implicated by *in-vivo* rodent bioassays as carcinogens increased sister chromatid exchanges when tested as gases at anesthetizing concentrations. These included chloroform,[12] trichloroethylene,[13] and isoflurane.[1] Halothane, ni-

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TABLE 1. Conditions of Exposure to Anesthetics and Resulting Sister Chromatid Exchanges

	Delivered Dose (Per Cent, v/v)	Percentage of Delivered Dose Remaining after One Hour of Exposure	Percentage of Delivered Dose Remaining after 24 Hours of Exposure	Sister Chromatid Exchanges per Chromosome ± SE after One Hour of Exposure
Control A	—	—	—	0.536 ± 0.018
Nitrous oxide	75.	96	80	0.457 ± 0.017
Diethyl ether	1.97	75		0.513 ± 0.017
Trichloroethylene	0.17	59		0.514 ± 0.018
Halothane	0.79	87	84	0.519 ± 0.021
Enflurane	1.70	82	85	0.534 ± 0.021
Chloroform	0.71	78		0.544 ± 0.018
Divinyl ether	1.99	76		0.706 ± 0.022*,†
Fluroxene	2.47	89	92	1.147 ± 0.030*,‡
Ethyl vinyl ether	1.79	98		1.338 ± 0.031*
Control B	—	—	—	0.597 ± 0.019
Isoflurane	11.3	85		0.621 ± 0.020
Isoflurane	1.13	90	90	0.641 ± 0.018
Methoxyflurane	1.19	70		0.550 ± 0.020
Methoxyflurane	0.20	72	43	0.627 ± 0.019

* Differs from Control A, $P < 0.01$.
† Differs from fluroxene, ethyl vinyl ether, $P < 0.01$.
‡ Differs from ethyl vinyl ether, $P < 0.01$.

trous oxide, enflurane, methoxyflurane and diethyl ether also did not increase exchanges. However, the vinyl-containing compounds fluroxene, ethyl vinyl ether, and divinyl ether all increased exchanges. The less stable vinyl group is more susceptible to epoxide formation than organic ethers, which contain a saturated halogenated hydrocarbon grouping or the halogenated hydrocarbons themselves. We suspect that it is this component that leads to mutagenicity. Vinyl-containing compounds (*e.g.*, vinyl chloride) are mutagens[14] and carcinogens.[15]

We believe that the 1-MAC levels we used assured us of producing the maximum effects of any metabolites of these drugs. Other investigators[16,17] have shown that metabolic enzymes are saturated at concentrations very much lower than 1 MAC. For example, Sawyer *et al.* showed maximal hepatic extraction in miniature swine at halothane, 0.01 MAC.

Our findings are similar to those of Baden *et al.*[5] Both the sister chromatid exchange technique and the Ames assay give positive responses for fluroxene. The Ames test uses bacterial cells rather than mammalian cells and studies the appearance of frame shift and base-pair reversion mutations in the specially prepared *Salmonella* strains, whereas sister chromatid exchanges involve double-strand DNA exchanges between parent and daughter polynucleotide strands. Thus, both tests involve chromosomal material, but the genetic endpoints are different. Both the Ames test and the test of sister chromatid exchanges measure alterations in DNA. Changes in DNA could interfere with mechanisms controlling cellular growth. Lack of such control results in cancer. More than 300 chemicals have been subjected to the Ames assay; 90 per cent of known carcinogens proved positive, while only 13 per cent were falsely positive.[19] Perry and Evans[6] have studied 15 proven (or suspected) mutagen–carcinogens. These alkylating and intercalating agents were efficient inducers of sister chromatid exchanges. Such evidence suggests that these tests of the effects of chemicals on chromosomal DNA usually (but not invariably) predict the likelihood of carcinogenicity of a compound. That both tests give positive results with fluroxene and the CHO test is positive with divinyl and ethyl vinyl ethers suggests that any vinyl-containing anesthetic deserves thorough testing *in vivo* before human beings are exposed to it.

The results of our 24-hour exposures seem to indicate that any influence the currently used agents and fluroxene might have on the orderly process of mitosis is minimal in the absence of metabolism of the anesthetic.

The results of our study in mammalian cells suggest that older anesthetics containing a vinyl moiety may be mutagen–carcinogens, but that no currently used anesthetic is a mutagen. If current inhaled anesthetics are carcinogenic, at worst they are weakly so. Our results provide additional evidence that patients and operating room personnel are at little risk from anesthesia-induced cancer.

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