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Enflurane Effects on Cell Division and Macromolecular Synthesis in Tetrahymena pyriformis:

Comparison with Halothane

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The objectives of this study were to determine the effects of enflurane on cell division and incorporation of macromolecular precursors into nucleic acid of *Tetrahymena pyriformis*, to determine the effects of both enflurane and halothane on incorporation of amino acids into proteins of *T. pyriformis*, and to compare the effects of these two anesthetics using these data and data previously reported for halothane. After exposure for three hours, cell division was inhibited 66 and 81 per cent by enflurane, 2.2 and 4.3 per cent, respectively. Similarly, exposure for three hours resulted in 25 and 81 per cent inhibition of ^{14}C-thymidine incorporation and 74 and 98 per cent inhibition of ^{14}C-uridine incorporation by enflurane, 2.2 and 4.3 per cent, respectively. Enflurane, as previously shown for halothane, did not inhibit DNA or RNA synthesis in isolated nuclei (assayed using ^{3}H-labeled nucleoside triphosphates as precursors). Finally, incorporation of ^{14}C-amino acids was inhibited 17 and 60 per cent by exposure for three hours to halothane (1.2 and 2.4 per cent, respectively) and 49 and 77 per cent by exposure for three hours to enflurane (2.2 and 4.3 per cent, respectively). The comparison of halothane and enflurane was made to determine whether the effects on *T. pyriformis* were proportional to the absolute (per cent or molar) anesthetic concentration, or to MAC (minimum alveolar concentration). It was found that inhibition of cell division is proportional to MAC (*i.e.*, equivalent inhibition is observed when the anesthetic concentration in the culture medium is the same as that in blood exposed to either 1 MAC halothane or 1 MAC enflurane), and that inhibition of macromolecular precursor incorporation (in intact cells) is proportional to the absolute (per cent or molar) anesthetic concentration. It is concluded that inhibition of cell division by the anesthetics is not due to direct inhibition of nucleic acid synthesis, nor is it directly related to inhibition of thymidine, uridine, or amino acid incorporation in intact cells. (Key words: Anesthetics, volatile: halothane; enflurane. Cells, replication, thymidine; uridine; amino acids. Metabolism: DNA; protein; RNA.)

HALOTHANE has been shown to inhibit cell division[1–5] and incorporation of precursors into DNA,[5–10] RNA,[5,11] and protein[10,11] of eukaryotic cells *in vitro*. Comparable studies have not been done with enflurane, although it does inhibit division of *Euglena gracilis*.[4]

The objectives of the present study were to determine the effects of enflurane on cell division and incorporation of macromolecular precursors into nucleic acids of *Tetrahymena pyriformis*, to determine the effects of both enflurane and halothane on incorporation of amino acids into proteins of *T. pyriformis*, and to compare the effects of these two anesthetics using these data and data previously reported for halothane.[5]

Materials and Methods

Cultures of *Tetrahymena pyriformis*, strain GL, were maintained in Hogg's medium (stock cultures), and grown to logarithmic growth phase in 2 per cent proteose peptone–liver extract medium as described previously.[12] These cultures were exposed to enflurane (dispensed from an Ethrane® vaporizer) or halothane (dispensed from a Fluotec 3 vaporizer) with a carrier gas flow (air) of 2 l/min. The gas was passed through a plexiglass exposure chamber (700 ml capacity) containing the cell culture or reaction mixture in a petri dish. The exposure chamber was a cylinder 4 cm high and 15 cm in diameter with a gas inlet and outlet, a sealed bottom, a sealed but removable top, and three sampling ports in the top with removable covers. In all experiments, a control (cell culture or reaction mixture) within an identical exposure chamber was exposed to a 2 l/min flow of air. Both exposure chambers received air from the same compressed air cylinder delivered through a Y-connector to identical flowmeters, and then to the gas inlet of the control chamber or through the anesthetic vaporizer to the gas inlet of the experimental chamber. Both chambers were placed on the same rotary mixer at 60 rpm during experiments, and were maintained at 27 C. Enflurane and halothane concentrations of gas delivered from the vaporizers were determined by gas chromatography using a Perkin-Elmer 900 gas chromatograph with a 6-foot Poropak Q® column.

To determine the effect of enflurane on cell division, 20 ml of a *T. pyriformis* culture (3–5 × 10^4 cells/ml) were placed in a sterilized petri dish (100 mm diameter) within each exposure chamber. The chambers were closed, the gas flows started to the control and experimental chambers, and duplicate cell samples of 0.5 ml removed from each chamber after a 15-min equilibration period between the gas and the

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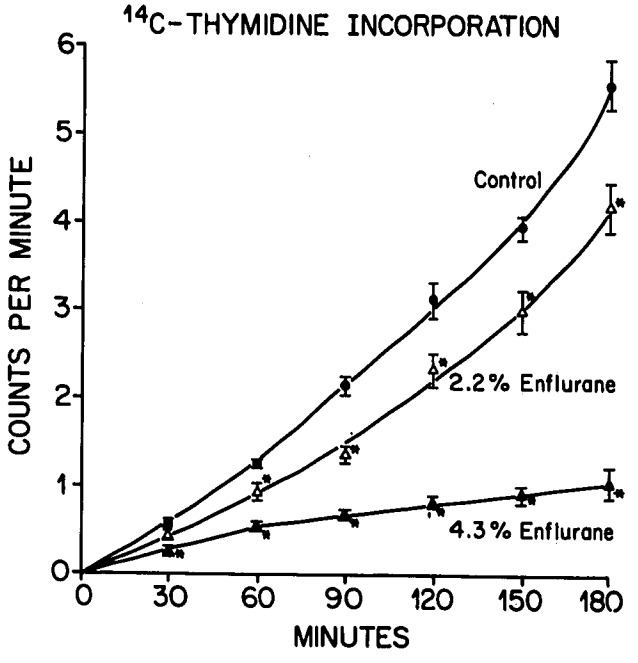

FIG. 1. Effects of enflurane on incorporation of ^{14}C-thymidine by *Tetrahymena pyriformis*. Cell cultures (2–3 × 10^4 cells/ml) were exposed to air (*closed circles*) or enflurane at 2.2 (*open triangles*) or 4.3 per cent (*closed triangles*) in air, and incorporation of 2-^{14}C-thymidine determined by the filter paper disc technique. Vertical bars indicate ±SEM. Asterisks indicate significant difference from control ($P < 0.05$). For each determination, n = 8 for control samples and n = 4 for enflurane-treated samples. Counts per minute are in thousands per 2.5 × 10^3 cells.

culture medium. Subsequent cell samples were taken at 30-min intervals for three hours. The cells were fixed in a formalin solution and the cell population determined using a Sedgwick-Rafter counting chamber.[12] Enflurane concentrations were determined at time zero and at hourly intervals.

To determine the effect of enflurane on nucleic acid precursor incorporation by intact cells, *T. pyriformis* was grown to a population of 2–3 × 10^4 cells/ml and a 20-ml aliquot of the culture placed in a sterilized petri dish (100 mm diameter) within each exposure chamber. Gas flows were then started to the control and experimental chambers. In each chamber, after 15 min of equilibration, a 3-ml sample of the cell culture was taken from the original 20-ml aliquot and placed in another petri dish (sterilized, 35 mm diameter) within that chamber. This second petri dish contained either 5 μCi (0.05 ml) of (2-^{14}C)-thymidine (55.7 mCi/mmol‡) or 5 μCi (0.05 ml) of (2-^{14}C)-uridine (55.6 mCi/mmol‡). At time zero and at 30-min intervals for three hours, duplicate 0.1-ml samples were withdrawn from each petri dish and processed by the filter paper disc procedure of Byfield and Scherbaum[13] to determine precursor incorporation. Radioactive counting of the discs was done with a Beckman model LS330 liquid scintillation counter using a scintillation fluid composed of 2,5-diphenyloxazole (PPO), 5 g/l of toluene. Cell counts and anesthetic concentrations were determined at hourly intervals during each experiment.

To determine the effects of halothane and enflurane on amino acid incorporation into protein by intact cells, *T. pyriformis* was grown to a population of 2–3 × 10^4 cells/ml and 20-ml aliquots of the culture placed in sterilized petri dishes (100 mm diameter) within the control (air) and the experimental (halothane or enflurane) exposure chambers. In each chamber, after 15 min of equilibration with the gas flows on, a 3-ml sample of the cell culture was taken from the original 20-ml aliquot and placed in another petri dish (sterilized, 35 mm diameter) within that chamber. This second petri dish contained 5 μCi (0.05 ml) of a ^{14}C-labeled amino acid mixture (algal protein hydrolysate, 1.87 mCi/mg‡). At time zero and at

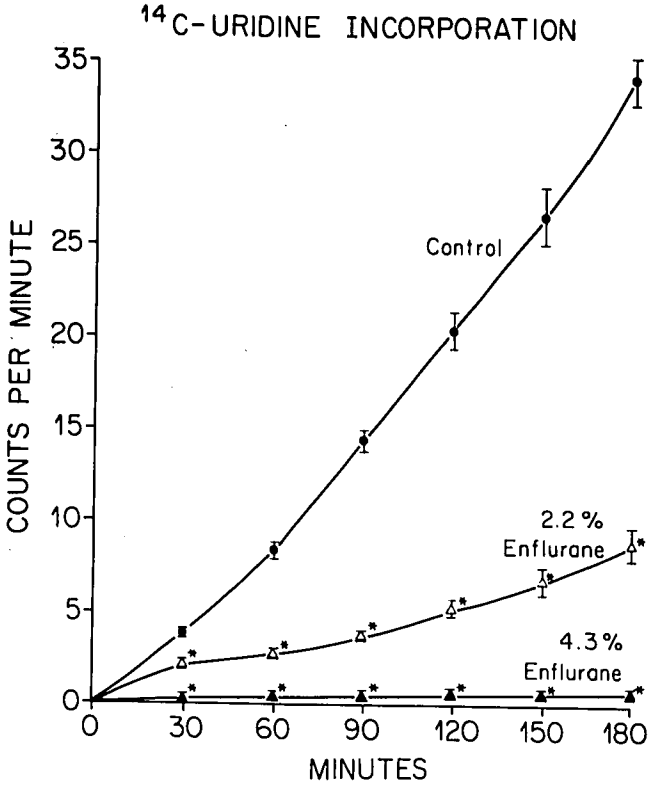

FIG. 2. Effects of enflurane on incorporation of ^{14}C-uridine by *Tetrahymena pyriformis*. Cell cultures (2–3 × 10^4 cells/ml) were exposed to air (*closed circles*) or enflurane at 2.2 (*open triangles*) or 4.3 per cent (*closed triangles*) in air, and incorporation of 2-^{14}C-uridine determined by the filter paper disc technique. Vertical bars indicate ±SEM. Asterisks indicate significant difference from control ($P < 0.05$). For each determination, n = 8 for control samples and n = 4 for enflurane-treated samples. Counts per minute are in thousands per 2.5 × 10^3 cells.

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30-min intervals for three hours, duplicate 0.1-ml samples were withdrawn from each petri dish and processed by the filter paper disc procedure of Byfield and Scherbaum[13] to determine amino acid incorporation. Scintillation counting was done as described above. Cell counts and anesthetic concentrations were done at hourly intervals during each experiment.

Finally, to determine the effect of enflurane on nucleic acid precursor incorporation by isolated nuclei, macronuclei were isolated from *T. pyriformis* by the procedure of Lee and Scherbaum.[14] DNA and RNA synthesis were assayed by incorporation of radioactivity from (methyl-^{3}H)-thymidine 5'-triphosphate (^{3}H-TTP) and (5-^{3}H)-uridine 5'-triphosphate (^{3}H-UTP), respectively, as described by Conklin and Chou.[15] The suspension of nuclei (5 ml in a 60-mm diameter petri dish) and the assay reaction mixture[15] (1 ml in a 35-mm diameter petri dish) containing either 2 μCi of ^{3}H-TTP (62.6 Ci/mmol§) or 2 μCi of ^{3}H-UTP (19.2 Ci/mmol§) were placed in each exposure chamber. Gas flows were then started to the control and experimental chambers and the solutions allowed to equilibrate for 15 min. The reaction was then started by addition of 1 ml of the suspension of nuclei to the petri dish containing the assay reaction mixture. Duplicate samples of 0.1 ml were withdrawn from each reaction mixture at time zero and at 10, 20, and 30 min (30 min reaction time allows maximum precursor incorporation[15]) and incorporation of precursors into DNA and RNA determined by the filter paper disc procedure.[13] Scintillation counting was done as described above. Anesthetic concentrations were determined at time zero and at 30 min.

Anesthetic concentrations of equivalent clinical potencies, *i.e.* concentrations equivalent to 0.5, 1, and 2 MAC (minimum alveolar concentration), were used to compare the effects of halothane and enflurane on the various cell processes. For halothane (MAC = 0.77 per cent, blood–gas partition coefficient = 2.3) the partition coefficient between the culture medium and air, determined by the procedure of Fink and Morikawa,[16] is 1.45 (standard error ± 0.03, n = 6). Therefore, to achieve the same anesthetic concentrations as those in blood exposed to 0.5, 1, or 2 MAC halothane, the culture medium was exposed to 0.6, 1.2, or 2.4 per cent halothane (0.77 × 2.3/1.45 times 0.5, 1, or 2), respectively. For enflurane (MAC = 1.68 per cent, blood–gas partition coefficient = 1.9) the culture medium–gas partition coefficient is 1.48 (standard error ± 0.04, n = 6). Therefore, the culture medium was exposed to 1.1, 2.2, or 4.3 per cent enflurane (1.68

§ New England Nuclear, Boston, Massachusetts 02118.

TABLE 1. Effects of Enflurane on Cell Division of *Tetrahymena pyriformis**

	Relative Cell Population†		
	1 Hour	2 Hours	3 Hours
Control	1.31 ± 0.03	1.63 ± 0.05	2.07 ± 0.04
Enflurane			
1.1 per cent	1.17 ± 0.05	1.49 ± 0.05	1.92 ± 0.10
2.2 per cent	1.21 ± 0.04	1.26 ± 0.06‡	1.36 ± 0.04‡
4.3 per cent	1.18 ± 0.09	1.10 ± 0.12‡	1.20 ± 0.11‡

* The initial cell population was 3–5 × 10^4 cells/ml. Cell cultures were exposed to air (control) or enflurane, 1.1, 2.2, or 4.3 per cent, in air, and cell counts were made at time zero and at hourly intervals.

† Mean ± SEM compared with initial cell count (time zero). A value of 1.0 represents no division, and a value of 2.0 represents a doubling of the cell population.

‡ Significant difference from control ($P < 0.05$); n = 12 for control samples, n = 4 for enflurane-treated samples.

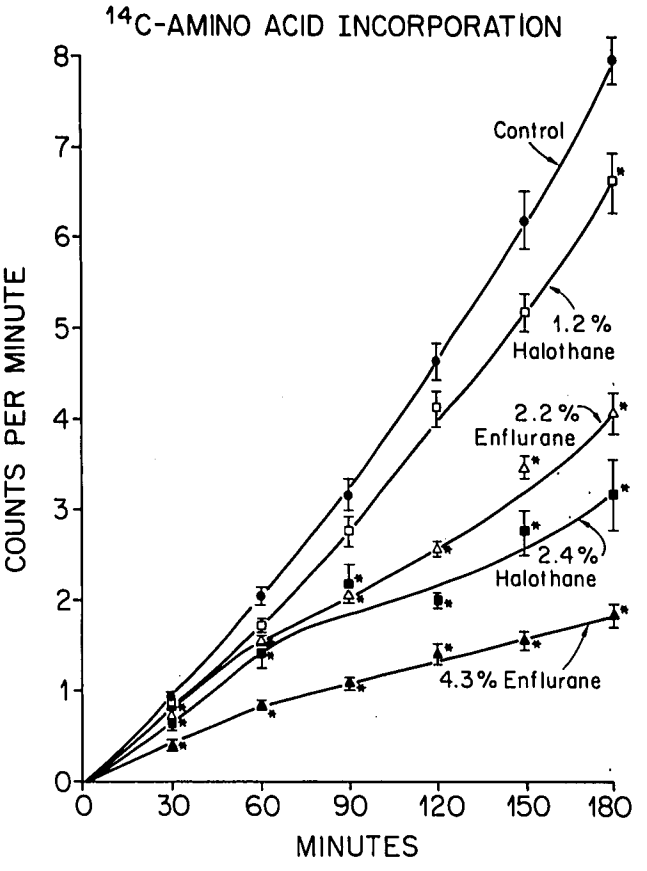

FIG. 3. Effects of halothane and enflurane on incorporation of ^{14}C-amino acids by *Tetrahymena pyriformis*. Cell cultures (2–3 × 10^4 cells/ml) were exposed to air (*closed circles*) or halothane at 1.2 (*open squares*) or 2.4 per cent (*closed squares*), or enflurane at 2.2 (*open triangles*) or 4.3 per cent (*closed triangles*) in air, and incorporation of ^{14}C-amino acids (algal protein hydrolysate) determined by the filter paper disc technique. Vertical bars indicate ±SEM. Asterisks indicate significant difference from control ($P < 0.05$). For each determination, n = 16 for control samples and n = 4 for halothane- and enflurane-treated samples. Counts per minute are in thousands per 2.5 × 10^3 cells.

TABLE 2. Effects of Enflurane on DNA Synthesis in Nuclei Isolated from *Tetrahymena pyriformis**

	CPM/10^6 Nuclei†		
	10 Min	20 Min	30 Min
Control	2,347 ± 152	2,964 ± 162	3,356 ± 242
Enflurane			
2.2 per cent	2,332 ± 164	3,195 ± 132	3,334 ± 59
4.3 per cent	2,413 ± 38	2,982 ± 67	3,438 ± 127
6.5 per cent	2,304 ± 209	3,198 ± 87	3,305 ± 42

* The assay reaction mixtures containing nuclei (0.5–1.0 × 10^6/ml) were exposed to air (control) or enflurane, 2.2, 4.3, or 6.5 per cent, in air, and incorporation of radioactivity from methyl-^{3}H-thymidine 5'-triphosphate was determined by the filter paper disc technique.

† Mean counts per minute incorporated from methyl-^{3}H-thymidine 5'-triphosphate ± SEM. There was no significant difference ($P > 0.05$) between the control value and that for any enflurane-treated sample; n = 12 for control samples, n = 4 for enflurane-treated samples.

× 1.9/1.48 times 0.5, 1, or 2) to achieve anesthetic concentrations equivalent to those in blood exposed to 0.5, 1, or 2 MAC enflurane, respectively.

Each experiment was performed a total of four times, with the individual experiments for each set being determined on a different day. All samples for cell counts and isotope incorporation were done in duplicate (from the same petri dish) and the average of the two values used for statistical analysis. Statistical analysis of the data was done using Student's *t* test for unpaired data, accepting $P < 0.05$ as significant.

Results

Enflurane, 1.1 per cent, had no effect on cell division of *Tetrahymena pyriformis* (table 1). Cell division was significantly inhibited by enflurane, 2.2 and 4.3 per cent, after two and three hours of exposure. After exposure for three hours, cell division was inhibited 66 and 81 per cent by enflurane, 2.2 and 4.3 per cent, respectively.

Exposure to enflurane, 2.2 or 4.3 per cent, significantly inhibited incorporation of 2-^{14}C-thymidine (except at 30 min, 2.2 per cent enflurane) and 2-^{14}C-uridine throughout the three hours of exposure (figs. 1 and 2). At three hours, thymidine incorporation was inhibited 25 and 81 per cent, and uridine incorporation was inhibited 74 and 98 per cent, by enflurane concentrations of 2.2 and 4.3 per cent, respectively.

Exposure to halothane, 2.4 per cent, or enflurane, 2.2 and 4.3 per cent, significantly inhibited incorporation of ^{14}C-amino acids throughout the three hours of exposure (fig. 3). Halothane, 1.2 per cent, significantly inhibited ^{14}C-amino acid incorporation only at three hours. Inhibitions at three hours were 17 and 60 per cent for halothane (1.2 and 2.4 per cent, respectively), and 49 and 77 per cent for enflurane (2.2 and 4.3 per cent, respectively).

Enflurane had no significant effect on DNA or RNA synthesis (measured by incorporation of radioactivity from methyl-^{3}H-thymidine 5'-triphosphate or 5-^{3}H-uridine 5'-triphosphate, respectively) in nuclei isolated from *T. pyriformis* (tables 2 and 3).

Comparing the effects of equivalent clinical (MAC) concentrations of halothane (from a previous study[5]) and enflurane on cell division at one, two, and three hours demonstrated no significant difference except for the one-hour samples exposed to 0.5 MAC of the anesthetics (table 4). However, exposures to enflurane resulted in significantly greater inhibitions of amino acid incorporation (1 and 2 MAC) and of thymidine and uridine incorporation (2 MAC only) than did exposures to equivalent clinical concentrations of halothane (table 5, halothane effects on thymidine and uridine incorporation from a previous study[5]).

Discussion

Both halothane[5] and enflurane inhibit cell division and incorporation of nucleic acid precursors, by intact cells, into DNA and RNA of *Tetrahymena pyriformis*. However, neither anesthetic inhibited DNA or RNA synthesis when these processes were studied in isolated nuclei using the nucleoside triphosphates as precursors. As previously discussed, these results suggest that inhibition of precursor (thymidine and uridine) incorporation into nucleic acids by intact cells is due to an effect of the anesthetics at a locus other than the DNA and RNA polymerase reactions.[5]

TABLE 3. Effects of Enflurane on RNA Synthesis in Nuclei Isolated from *Tetrahymena pyriformis**

	CPM/10^6 Nuclei†		
	10 Min	20 Min	30 Min
Control	9,562 ± 345	14,200 ± 583	16,194 ± 521
Enflurane			
2.2 per cent	9,681 ± 331	13,637 ± 646	15,509 ± 552
4.3 per cent	9,185 ± 336	13,105 ± 159	15,794 ± 601
6.5 per cent	10,624 ± 445	14,579 ± 626	16,868 ± 853

* The assay reaction mixtures containing nuclei (0.5–1.0 × 10^6/ml) were exposed to air (control) or enflurane, 2.2, 4.3, or 6.5 per cent, in air, and incorporation of radioactivity from 5-^{3}H-uridine 5'-triphosphate was determined by the filter paper disc technique.

† Mean counts per minute incorporated from 5-^{3}H-uridine 5'-triphosphate ± SEM. There was no significant difference ($P > 0.05$) between the control value and that for any enflurane-treated sample; n = 12 for control samples, n = 4 for enflurane-treated samples.

Possible mechanisms of action of the anesthetics are inhibition of a) nucleoside phosphorylation to the nucleoside triphosphates, b) intracellular transport of the precursors to their sites of incorporation, or c) cellular energy-generating systems. Inhibition of membrane transport of the precursors would also inhibit their incorporation by intact cells, although this does not appear to be the mechanism for inhibition of thymidine incorporation by halothane.[6,17]

Exposure to halothane and enflurane also resulted in a dose-dependent inhibition of amino acid incorporation. Determination of the site of anesthetic action on this process must await further investigation.

Comparison of halothane and enflurane effects on cell division demonstrated no significant difference (except in the one-hour samples exposed to 0.5 MAC) between equivalent clinical concentrations of the two anesthetics (*i.e.*, equivalent inhibitions of cell division are observed when the anesthetic concentration in the culture medium is the same as that in blood exposed to either 1 MAC halothane or 1 MAC enflurane). Since the absolute (per cent of molar) concentration of enflurane is approximately twice that of halothane for an equivalent clinical concentration, these results suggest that inhibition of cell division is proportional to MAC and not to the absolute anesthetic concentration. However, at culture medium concentrations equivalent to those in blood exposed to 1 and 2 MAC halothane or enflurane, enflurane exposure resulted in significantly greater inhibitions of thymidine and uridine incorporation (2 MAC only) and of amino acid incorporation (both concentrations). In contrast to the anesthetic effect on cell division, these results suggest that inhibition of macromolecular precursor incorporation (in intact cells) is proportional to the absolute (per cent or molar) anesthetic concentration instead of to MAC. This conclusion is also supported by the difference between the effects of 1 MAC halothane and 1 MAC enflurane on thymidine incorporation, *i.e.*, significant inhibition by enflurane throughout the three hours of exposure (except at 30 min), whereas significant inhibition by halothane was observed for the three-hour sample only.[5]

Finally, since inhibition of cell division appears to be proportional to MAC, whereas inhibition of precursor incorporation (in intact cells) is proportional to the absolute anesthetic concentration, the effect on cell division appears not to be directly related to the inhibition of thymidine, uridine, or amino acid incorporation. The lack of inhibition of DNA or RNA synthesis in nuclei suggests also that the anesthetic effect on cell division is not due to direct inhibition of nucleic acid synthesis. Others have also suggested that inhibition of cell maturation by halothane is not related to effects on DNA synthesis (measured by *in vivo* precursor incorporation in rats),[18] and that inhibition of cell division by halothane is not related to effects on RNA or protein synthesis (measured by *in vitro* precursor incorporation of cell cultures).[1] Assuming this is true, other possible mechanisms of anesthetic action for inhibition of cell division are disruption of microfilaments[1] and disruption of microtubules.[19] In this regard, *T. pyriformis* possesses cytoplasmic and nuclear microtubules, and inhibition of nuclear microtubule

TABLE 4. Comparison of Halothane and Enflurane Effects on Cell Division of *Tetrahymena pyriformis**

	Relative Cell Population†		
	1 Hour	2 Hours	3 Hours
Halothane 0.5 MAC	1.38 ± 0.02	1.60 ± 0.02	1.97 ± 0.04
Enflurane 0.5 MAC	1.17 ± 0.05‡	1.49 ± 0.05	1.92 ± 0.10
Halothane 1 MAC	1.16 ± 0.04	1.24 ± 0.05	1.68 ± 0.14
Enflurane 1 MAC	1.21 ± 0.04	1.26 ± 0.06	1.36 ± 0.04
Halothane 2 MAC	1.20 ± 0.02	1.13 ± 0.01	1.34 ± 0.04
Enflurane 2 MAC	1.18 ± 0.09	1.10 ± 0.12	1.20 ± 0.11

* Halothane data are from Conklin and Lau,[5] corrected for the difference between the halothane and enflurane controls. Enflurane data are from table 1. Exposure to anesthetic concentrations 0.5, 1, and 2 MAC (0.6, 1.2, and 2.4 per cent halothane or 1.1, 2.2, and 4.3 per cent enflurane, respectively) results in anesthetic concentrations in the culture medium equivalent to those of blood exposed to 0.5, 1, and 2 MAC of the anesthetic.

† The mean relative cell population ± SEM compared with the initial cell count (time zero). A value of 1.0 represents no division, and a value of 2.0 represents a doubling of the cell population.

‡ Significant difference ($P < 0.05$) between the values for halothane and enflurane; n = 4 for all samples.

TABLE 5. Comparison of Halothane and Enflurane Effects on Incorporation of Macromolecular Precursors by *Tetrahymena pyriformis**

	Precursor Incorporation: Per Cent of Control†		
	Thymidine	Uridine	Amino Acids
Halothane 1 MAC	80.0 ± 3.6	28.5 ± 6.4	82.9 ± 4.2
Enflurane 1 MAC	74.9 ± 5.1	25.9 ± 2.8	50.9 ± 2.9‡
Halothane 2 MAC	58.2 ± 12.6	8.4 ± 1.4	39.5 ± 5.3
Enflurane 2 MAC	18.6 ± 3.4‡	2.2 ± 0.2‡	22.7 ± 2.0‡

* Halothane values for thymidine and uridine were calculated from data of Conklin and Lau.[5] Other values were calculated from data in figures 1–3. Exposure to anesthetic concentrations 1 and 2 MAC (1.2 and 2.4 per cent halothane or 2.2 and 4.3 per cent enflurane, respectively) result in anesthetic concentrations in the culture medium equivalent to those of blood exposed to 1 and 2 MAC of the anesthetic.

† Precursor incorporations by the halothane- and enflurane-exposed cultures (3 hour samples) expressed as mean percentages of control ± SEM.

‡ Significant difference ($P < 0.05$) between the values for halothane and enflurane; n = 4 for all samples.

formation or disruption of these structures will inhibit cell division of this organism.[20]

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