

Halothane Does Not Inhibit Synthesis of Nucleic Acids in *Tetrahymena pyriformis*

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The effect of halothane on precursor incorporation into nucleic acids was studied in *Tetrahymena pyriformis*, a ciliate protozoan. At concentrations that blocked cell division (1.2 and 2.4 per cent), halothane inhibited incorporation of ^{14}C -thymidine and ^{14}C -uridine into DNA and RNA, respectively, in intact cells. However, in nuclei isolated from *T. pyriformis*, the anesthetic did not inhibit DNA and RNA synthesis when these processes were assayed using the nucleoside triphosphates (^3H -thymidine triphosphate and ^3H -uridine triphosphate) as precursors. It is concluded that halothane does not directly inhibit nucleic acid synthesis (i.e., the nucleic acid polymerase reactions), and that the inhibition of precursor incorporation observed in intact cells is due to an effect at a locus other than the DNA and RNA polymerase reactions. (Key words: Anesthetics, volatile: halothane. Cells, replication: thymidine; uridine. Metabolism: DNA; RNA. Toxicity.)

HALOTHANE has been shown to inhibit the incorporation of nucleic acid precursors into DNA and RNA by mammalian cells *in vitro*,¹⁻⁵ although some investigators have observed only minimal effects on these processes.⁶ This inhibition of nucleic acid synthesis may account for the interference with interphase of the cell cycle, as halothane has been shown to prolong the G₁ (pre-DNA synthesis) phase,⁵ the S (DNA synthesis) phase,⁷ and the G₂ (post-DNA synthesis) phase.⁸ Halothane also interferes with the division process in *Vicia faba*,⁹ in which this agent produces c-mitosis. These effects, which alter the cell's progression through the cell cycle (interphase and mitosis), probably account for the inhibition of cell growth by halothane.^{8,10,11}

This study was designed to evaluate further the effect of halothane on DNA and RNA synthesis. The organism used for this investigation was the ciliate protozoan *Tetrahymena pyriformis*, a eucaryotic organism possessing a nucleus and other subcellular organelles similar to those of mammalian cells.¹² The objectives were to determine the effects of halothane on cell division and incorporation of nucleic acid precursors into DNA and RNA by intact cells and isolated nuclei.

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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.¹³ These cultures were exposed to halothane, which was 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 halothane 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. Halothane concentrations of gas delivered from the vaporizer were determined by gas chromatography using a Perkin-Elmer 900 gas chromatograph with a 6-foot Poropak Q® column.

To determine the effect of halothane on cell division, 20 ml of a *T. pyriformis* culture ($3-5 \times 10^4$ 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 cell samples of 0.5 ml removed from each chamber after a 15-min equilibration period between the gas and the 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 Sedgewick-Rafter counting chamber.¹³ Halothane concentrations were determined at time zero and at hourly intervals.

To determine the effect of halothane on nucleic acid precursor incorporation by intact cells, *T. pyriformis* was grown to a population of $2-3 \times 10^4$ 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 μ Ci (0.05 ml) of (2- 14 C)-thymidine (55.7 mCi/mmol \pm) or 5 μ Ci (0.05 ml) of (2- 14 C)-uridine (55.6 mCi/mmol \pm). 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 Scherbaum¹⁴ 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.

Finally, to determine the effect of halothane on nucleic acid precursor incorporation by isolated nuclei, macronuclei were isolated from *T. pyriformis* by the procedure of Lee and Scherbaum.¹⁵ 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.¹⁶ The suspension of nuclei (5 ml in a 60-mm diameter petri dish) and the assay reaction mixture¹⁶ (1 ml in a 35-mm diameter petri dish) containing either 2 μ Ci of 3 H-TTP (62.6 Ci/mmol \pm) or 2 μ Ci of 3 H-UTP (19.2 Ci/mmol \pm) were placed in each exposure chamber.

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

	Relative Cell Population†		
	1 Hour	2 Hours	3 Hours
Control	1.27 \pm 0.03	1.63 \pm 0.05	1.95 \pm 0.05
Halothane			
0.6 per cent	1.34 \pm 0.02	1.60 \pm 0.02	1.86 \pm 0.04
1.2 per cent	1.12 \pm 0.04‡	1.24 \pm 0.05‡	1.58 \pm 0.13‡
2.4 per cent	1.16 \pm 0.02‡	1.13 \pm 0.01‡	1.26 \pm 0.04‡

* The initial cell population was $3-5 \times 10^4$ cells/ml. Cell cultures were exposed to air (control) or halothane at 0.6, 1.2 or 2.4 per cent in air, and cell counts were made at time zero and at hourly intervals.

† Value \pm 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 halothane samples.

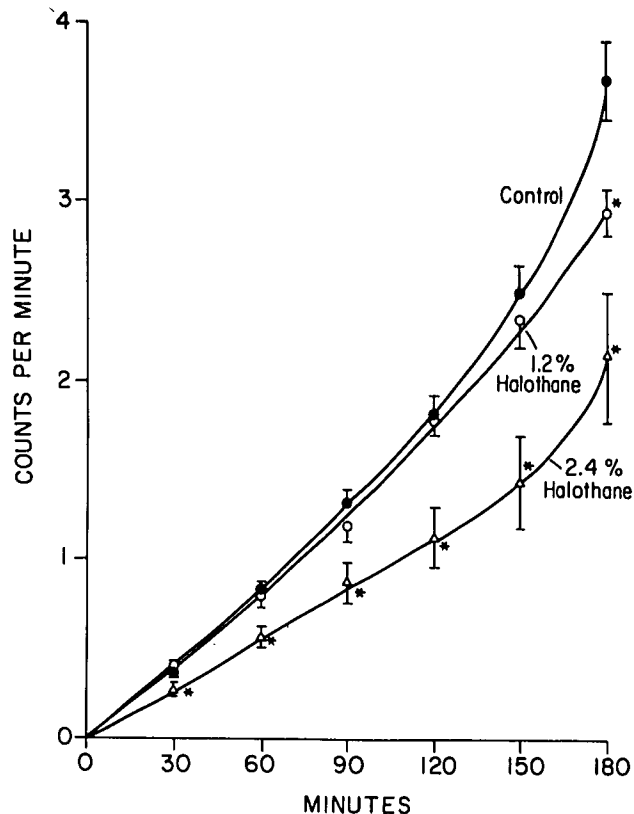


FIG. 1. Effects of halothane on incorporation of 14 C-thymidine by *Tetrahymena pyriformis*. Cell cultures ($2-3 \times 10^4$ cells/ml) were exposed to air (closed circles) or halothane at 1.2 (open circles) or 2.4 per cent (open triangles) in air, and incorporation of (2- 14 C)-thymidine determined by the filter paper disc technique. Vertical bars indicate \pm SEM. Asterisks indicate significant difference from control ($P < 0.05$). The n for each determination was 8 for control samples and 4 for halothane samples. Counts per minute are in thousands per 2.5×10^3 cells.

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 incorporation¹⁶) and incorporation of precursors into DNA and RNA determined by the filter paper disc procedure.¹⁴ Scintillation counting was done as described above. Anesthetic concentrations were determined at time zero and at 30 min.

Statistical analysis of the data was done using a t test for unpaired data, accepting $P < 0.05$ as significant.

Results

Halothane, 0.6 per cent, had no effect on cell division of *Tetrahymena pyriformis* (table 1). Cell division

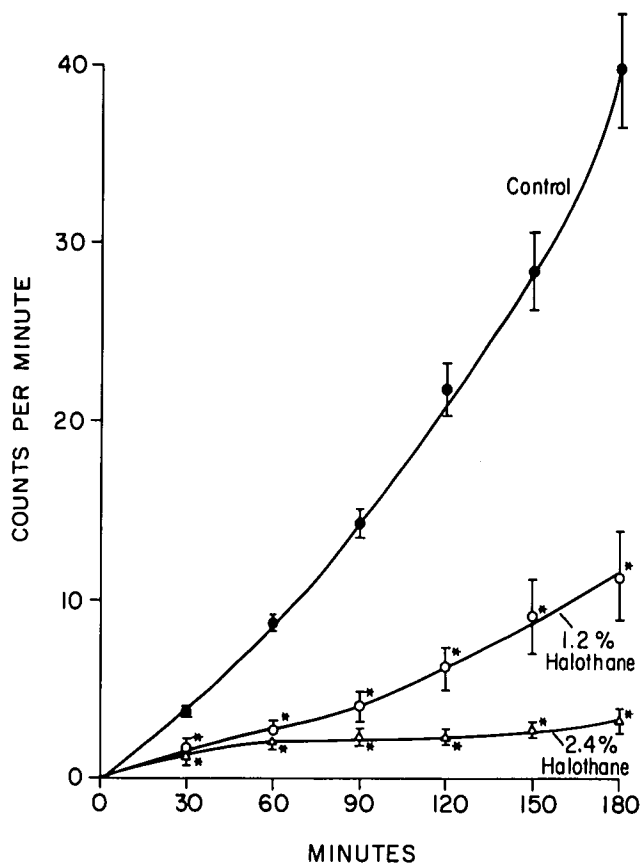


FIG. 2. Effects of halothane on incorporation of ^{14}C -uridine by *Tetrahymena pyriformis*. Cell cultures ($2-3 \times 10^4$ cells/ml) were exposed to air (closed circles) or halothane at 1.2 (open circles) or 2.4 per cent (open triangles) in air and incorporation of (^{14}C)-uridine determined by the filter paper disc technique. Vertical bars indicate \pm SEM. Asterisks indicate significant difference from control ($P < 0.05$). The n for each determination was 8 for control samples and 4 for halothane samples. Counts per minute are in thousands per 2.5×10^3 cells.

was significantly inhibited by halothane at 1.2 and 2.4 per cent.

Exposure to halothane, 1.2 per cent, resulted in significant inhibition of (^{14}C)-thymidine incorporation only after three hours (fig. 1). Exposure to halothane, 2.4 per cent, resulted in significant inhibition of thymidine incorporation throughout the three hours of exposure. At three hours, the inhibition of thymidine incorporation by halothane, 1.2 or 2.4 per cent, was 20 or 42 per cent, respectively.

Exposure to halothane, 1.2 and 2.4 per cent, resulted in significant inhibition of (^{14}C)-uridine incorporation throughout the exposure period (fig. 2). At three hours uridine incorporation was inhibited by halothane, 1.2 and 2.4 per cent, 72 and 92 per cent, respectively.

Halothane had no effect on DNA synthesis in nuclei isolated from *T. pyriformis* except for the 30-min sample

exposed to halothane, 3.7 per cent (table 2), which showed a small but significant increase in DNA synthesis. Likewise, there was no significant inhibition of RNA synthesis by halothane at any of the concentrations studied (table 3).

Discussion

This study had three objectives. The first was to determine the effect of halothane on cell division of *Tetrahymena pyriformis*. Halothane significantly inhibited cell growth of this organism during exposure for one generation time (three hours). These results are similar to those obtained by other investigators who have studied the effects of halothane on *T. pyriformis*.¹⁷

The second objective was to determine the effects

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

	CPM/ 10^6 Nuclei†		
	10 Min	20 Min	30 Min
Control	2,022 \pm 86	2,684 \pm 68	3,027 \pm 40
Halothane			
1.2 per cent	2,227 \pm 133	2,858 \pm 83	2,942 \pm 45
2.4 per cent	2,004 \pm 206	2,609 \pm 103	2,980 \pm 78
3.7 per cent	1,860 \pm 26	2,664 \pm 75	3,169 \pm 47‡

* The assay reaction mixtures containing nuclei ($0.5-1.0 \times 10^6$ /ml) were exposed to air (control) or halothane at 1.2, 2.4 or 3.7 per cent in air, and incorporation of radioactivity from (methyl- ^3H)-thymidine 5'-triphosphate determined by the filter paper disc technique.

† Counts per minute incorporated from (methyl- ^3H)-thymidine 5'-triphosphate \pm SEM.

‡ Significant difference from control ($P < 0.05$), $n = 12$ for control samples, $n = 4$ for halothane samples.

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

	CPM/ 10^6 Nuclei†		
	10 Min	20 Min	30 Min
Control	5,288 \pm 318	7,770 \pm 342	9,103 \pm 154
Halothane			
1.2 per cent	5,179 \pm 903	8,910 \pm 834	9,543 \pm 1,103
2.4 per cent	4,946 \pm 423	7,920 \pm 483	9,286 \pm 249
3.7 per cent	4,759 \pm 637	7,318 \pm 808	9,853 \pm 547

* The assay reaction mixtures containing nuclei ($0.5-1.0 \times 10^6$ /ml) were exposed to air (control) or halothane at 1.2, 2.4 or 3.7 per cent in air, and incorporation of radioactivity from (^3H)-uridine 5'-triphosphate determined by the filter paper disc technique.

† Counts per minute incorporated from (^3H)-uridine 5'-triphosphate \pm SEM.

‡ There was no significant difference ($P > 0.05$) between the control value and that for any halothane-treated sample, $n = 9$ for control samples, $n = 3$ for halothane samples.

of halothane on incorporation of nucleic acid precursors into DNA and RNA by intact cells. Halothane inhibited incorporation of the nucleosides thymidine and uridine, although the inhibition observed was greater with uridine than with thymidine. These results are similar to those observed by other investigators,¹⁻⁵ except for Ishii and Corbascio,⁶ who observed minimal effects of halothane on thymidine and uridine incorporation. Their results may have been due to inadequate control of the administered dose of halothane (liquid halothane added directly to a variably open system), as suggested by Jackson.¹ However, as many different processes are required for incorporation of nucleosides into DNA and RNA by intact cells, these results do not identify the site of action of halothane. For example, incorporation of these precursors into nucleic acids involves many processes, including membrane transport of the nucleosides into the cell, phosphorylation of the nucleosides to the nucleoside triphosphates, intracellular and intranuclear transport of the precursors to their sites of incorporation, and the DNA and RNA polymerase reactions themselves.

The third objective was to determine the direct effects of halothane on DNA and RNA synthesis, *i.e.*, the polymerase reactions. This was studied in isolated nuclei using the nucleoside triphosphates as precursors. These results demonstrate that halothane at 1.2, 2.4, and 3.7 per cent does not inhibit the nucleic acid polymerase reactions. The reason for the small increase in DNA synthesis at 30 min with halothane, 3.7 per cent, is unclear. Therefore, the inhibition of precursor (thymidine and uridine) incorporation into nucleic acids by intact cells appears to be due to an effect of halothane at some other locus, *e.g.*, phosphorylation of the nucleosides or intracellular transport of the precursors. Inhibition of membrane transport is unlikely, as halothane does not inhibit thymidine entry into mammalian cells.^{1,18} A mechanism that may explain the results observed with intact cells is the inhibition by halothane of aerobic energy-generating systems (*i.e.*, electron transport and oxidative phosphorylation in mitochondria) in the cell.¹⁹ As impaired cellular energetics would interfere with production of high-energy compounds necessary for phosphorylation of the nucleosides, these effects may account for the inhibition of thymidine and uridine incorporation.

Finally, the relevance of these results to effects of halothane on mammalian cells should be considered. As *T. pyriformis* possesses subcellular organelles and enzyme systems for macromolecular synthesis similar

to those of other eucaryotic cells,¹² the above-described findings suggest that halothane may not directly inhibit nucleic acid synthesis (the polymerase reactions) in mammalian cells. Confirmation of this, however, must await further study.

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