

Cultured Neuroblastoma Cells and Halothane:

Effects on Cell Growth and Macromolecular Synthesis

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Cultured mouse neuroblastoma cells were grown in air-CO₂ or air-CO₂-halothane-gassed incubators. In the presence of halothane the growth rate of the cells was inhibited in a dose-dependent manner; 2 per cent halothane completely inhibited cell growth, while at 0.3 per cent halothane, the growth rate was 74 per cent of the control rate. The biosynthesis of protein and RNA in cells grown in the control atmosphere and that in cells grown in 1 per cent halothane were compared by several techniques. No significant difference between the rates of synthesis of these two macromolecules could be detected. Furthermore, a comparison of labeled protein and RNA by SDS-polyacrylamide gel electrophoresis revealed no qualitative difference. From this and previous work it is concluded that halothane affects the morphology and growth rate of cultured mouse neuroblastoma cells by disrupting cytoplasmic actin-like microfilaments. (Key words: Anesthetics, volatile, halothane; Cells, multiplication; Metabolism, RNA.)

WE HAVE BEEN INTERESTED in defining the mechanism of action of the volatile anesthetic, halothane, on cells of neural origin. As a general anesthetic, halothane clearly must interfere with the transfer of neuronal information. The effects of halothane on intermediary metabolism have been studied.¹⁻⁴ While volatile anesthetics, including halothane, may cause a shift in intermediary metabolism from oxidative to glycolytic pathways, the energy available to a cell may be of more physiologic importance. Any alteration in the availability of ATP (or similar energy sources) might well be reflected in the synthesis and metabolism of cellular macromolecules.

Relatively little information about the effects of halothane (or other volatile anesthetics) on the synthesis of protein, RNA or DNA in cultured mammalian cells has been reported.⁵⁻⁸ These studies were usually carried out on suspension or spinner cultured cells. We previously reported that halothane inhibits the growth of mouse neuroblastoma cells in monolayer culture.^{9,10} This general observation has been confirmed recently in studies with different cell lines.^{11,12} In an earlier paper,¹³ we showed that low concentrations of halothane have profound, but readily reversible, effects on the

integrity of a 40-80-Å class of microfilaments in cultured mouse neuroblastoma cells. In this report we present studies in which we examined certain biochemical characteristics of the effects exerted by halothane on cultured mouse neuroblastoma cells.

Materials and Methods

Mouse neuroblastoma cells (clone NB2a) were used in all experiments. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, N. Y.) containing 10 per cent gammaglobulin-free newborn calf serum (Gibco). Stock cultures were maintained in large Falcon plastic flasks and 100-mm Falcon tissue-culture petri dishes. Cells were not recloned during the course of this work.

Cells were grown at 37 C in adjacent, identical incubators. The control atmosphere consisted of a flow of air-5% CO₂ (approximately 8 lpm), while the halothane incubator received the same air-CO₂ flow and halothane, the concentration of which was regulated with a Copper Kettle vaporizer (Foregger, Inc., New York). Gas concentrations were monitored as described in the preceding paper.¹³

MEASUREMENT OF GROWTH RATE

In a typical experiment, 12 100-mm Falcon tissue-culture dishes, each containing 25-30 ml of medium, were inoculated with 5×10^5 cells/dish and placed in the control incubator for 2-3 hours to allow the cells to attach. Six of the dishes were then transferred to a halothane-perfused incubator that had been pre-equilibrated with the desired concentration of halothane. Twenty-four, 48, and 72 hours after the initial plating of the cells, a pair of dishes was removed from each incubator and viokased; the cells were collected by centrifugation and resuspended in 2.0 ml of fresh medium. Duplicate samples of cells were counted from each replicate set on a hemocytometer using an inverted phase microscope. The cell suspensions had 10^5 to 3×10^7 cells/ml. Reproducibility of this method of counting was greater than 95 per cent.

LABELING OF CELLS WITH LEUCINE-³H OR URIDINE-³H

Similar experimental protocols were used to label cells with all radioactive precursors. Leucine-³H (31.9 Ci/mmol) was purchased from New England Nuclear Company (Boston, Mass.). Uridine-³H (24 Ci/mmol) was a product of Schwartz/Mann, Orange-

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Preliminary accounts of some of this work have appeared elsewhere.^{9,10,24}

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burg, N. Y. A few experiments were done with ^{14}C -labeled amino-acid hydrolysate (57 mCi/matom) purchased from Amersham Searle (Arlington Heights, Ill.).

For labeling studies, 60-mm Falcon tissue-culture dishes were used. The dishes were inoculated with approximately 5×10^5 cells/dish; they were placed in the control incubator overnight (16–20 hours) to allow the cells to attach and begin growing; then half the dishes were transferred to the halothane-equilibrated incubator. Sufficient dishes were inoculated so that two or three dishes were harvested at each time point for both control and experimental cultures. Experimental cells were grown in 1.0 per cent halothane-equilibrated atmospheres for 16–20 hours before labeling. Cells were labeled by adding leucine- ^3H to fresh control or halothane-equilibrated medium at an isotope concentration of 2–6 $\mu\text{Ci/ml}$. Growth medium was poured off and replaced by 2 ml of medium containing leucine- ^3H . At the desired times, the radioactive medium was poured off and the dishes immediately flushed with cold 5 per cent trichloroacetic acid (TCA). Cells were scraped off the dishes with a rubber policeman, transferred to centrifuge tubes, and sonicated. The cold TCA-insoluble material was collected by centrifugation. The precipitates were suspended in 1.0 ml of cold 5 per cent TCA; a sample (usually 0.1 ml) was removed for protein determination by the Lowry method.¹⁴ The remainder was collected on Whatman GF/C filters, washed with 10–20 ml cold 5 per cent TCA, rinsed in ethanol:ether (1:1), and finally washed with ether. The filters were dried under a heat lamp and counted in Liquifluor in a Packard liquid scintillation counter (Model 3385).

POLYACRYLAMIDE GEL ELECTROPHORESIS

Labeled protein samples from control and halothane cultures were examined by SDS-acrylamide gel electrophoresis according to the method of Weber and Osborn.¹⁵ The gels were stained with Coomassie blue according to the method of Fairbanks *et al.*¹⁶ and scanned at a wavelength of 560 nm in a Gilford 2400-S spectrophotometer equipped with a model 2410 linear transport. Uridine- ^3H -labeled RNA from control and experimental cultures was electrophoresed according to the method of Knowland¹⁷; gels were scanned at 260 nm. Both protein and RNA gels were frozen with liquid N_2 and sliced into 1-mm slices. The slices were soaked in 0.7 ml of 90 per cent NCS (Amersham Searle, Arlington Heights, Ill.) overnight and were counted in 10 ml of Liquifluor.

Results

EFFECTS OF HALOTHANE ON GROWTH RATE

The data in figure 1 show that halothane caused a substantial reduction in the growth rate of mouse

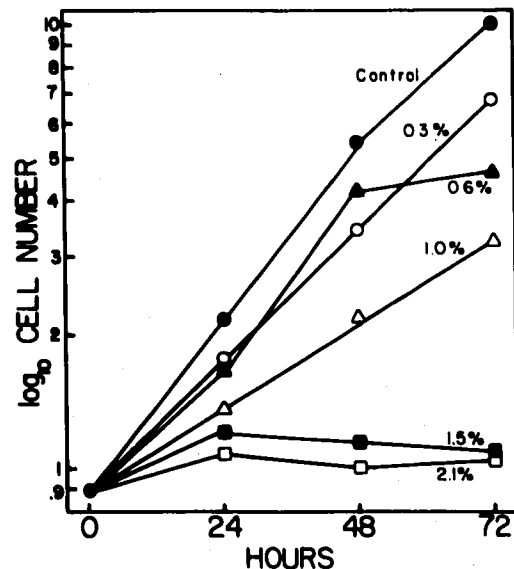


FIG. 1. Effect of halothane concentration on the growth rate of NB2a cells. The growth curves were carried out at the indicated concentrations of halothane in duplicate dishes for each time point. Each halothane concentration was tested twice. The actual cell numbers are normalized to the control growth rate; the latter growth rate represents the mean values from 17 different experiments. Control ●, 0.3 per cent halothane ○, 0.6 per cent ▲, 1 per cent △, 1.5 per cent ■, 2.1 per cent □.

neuroblastoma cells. Furthermore, it is evident that the inhibition was dose-dependent. The proportional reduction in growth rate was comparable to the proportional inhibition of neurite extension manifested by these cells over the same range of halothane concentrations.¹³

In order to plot cell-growth data in a single figure, the growth rate of cells grown at each halothane concentration was calculated as a percentage of the number of cells in the parallel control culture at the corresponding time. Furthermore, the slope of the control growth curve in figure 1 represents the average growth rate of many cultures grown in the control atmosphere. In the 17 growth curves measured, the doubling time of the mouse neuroblastoma cells was approximately 22 hours.

Cells grown in the presence of 1 per cent halothane grew at 50 per cent of the control growth rate. At this halothane concentration the proportion of cells with neurites was also half that of control cells (32 per cent *vs.* 68 per cent after 72 hours). Consequently, we chose 1 per cent halothane to be the concentration for radioisotope labeling experiments on neuroblastoma cells. Clinically, this concentration is sufficient to maintain light to moderate anesthesia.¹⁸

Figure 2 demonstrates that the effect of halothane on growth rate is reversible. Cells were allowed to grow in the control atmosphere or in the presence of 1 per cent halothane for 24 or 72 hours. In switching growth conditions, halothane-equilibrated medium was discarded and fresh control medium

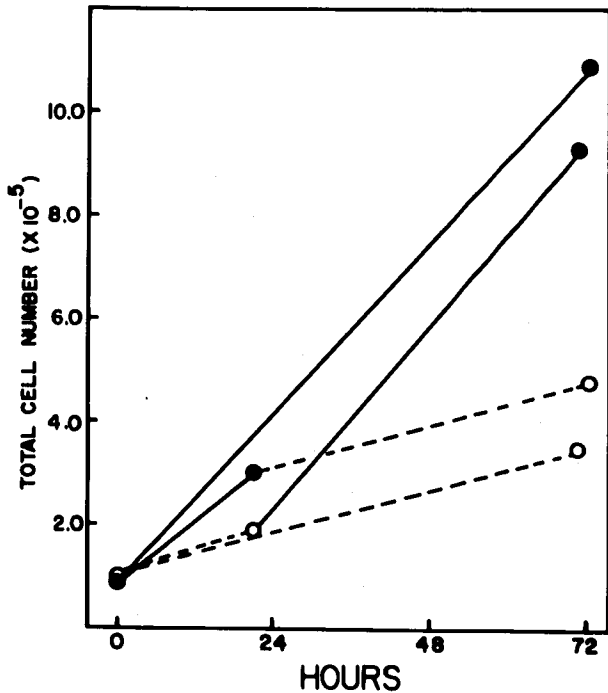


FIG. 2. Reversibility of halothane inhibition of growth rate of NB2a cells. Six 100-mm dishes were placed in a control incubator (—●—) and six in an incubator equilibrated with 1 per cent halothane (---○---). After 20 hours, the cells in two control and two halothane-grown dishes were counted, and two dishes from the control incubator were placed in the halothane incubator. At the same time two dishes were moved from the halothane to the control incubator, and all remaining dishes were counted after 72 hours.

was added to the dishes. The cells were allowed to grow for 48 hours more. The cells that were started in the control atmosphere were transferred to the halothane incubator. As is evident, the growth rates between 24 and 72 hours were virtually identical for cells in control and in halothane atmospheres, regardless of the growth condition during the first 24 hours of the experiment.

INCORPORATION OF LEUCINE-³H

In an attempt to explain the inhibitory effect of halothane on cell division, neurite extension, and microspike formation, we reasoned that halothane may interfere with the synthesis of macromolecules. Accordingly, we first examined the incorporation of leucine-³H into total cellular protein in the presence and absence of halothane. Table 1 presents data from two separate experiments. A time course of incorporation was routinely carried out to determine whether any difference between control and halothane cultures was due to differences in the kinetics of incorporation. The data suggest that halothane and control cultures incorporate leucine-³H into cold TCA-precipitable material to similar extents and with similar kinetics.

Figure 3 summarizes data from a large number of individual experiments. Each point represents a mean of at least six separate data points; some represent 14 separate points. These data show that the rate of leucine-³H incorporation into protein in halothane-grown cultures does not differ from that of control cultures.

Since the preceding experiments involved the addition of fresh medium (control or halothane-equilibrated) containing leucine-³H, it was possible that the similarity in rates during the two-hour pulse period reflected a "feeding" response of the cells.¹⁹ To evaluate this possibility, we collected the media (control and halothane) in which the cells had been grown, added leucine-³H to an appropriate volume, and labeled the cells in this "old" or "conditioned" medium. The results of this experiment (data not shown) were indistinguishable from those in the experiments described above.

In order to verify the lack of effect of halothane on leucine-³H incorporation, three additional types of experiments were done. First, cells were labeled in a long-term exposure to leucine-³H. Second, the degradation of cellular protein (prelabeled with leucine-³H) was measured. Finally, SDS-polyacrylamide gels were used to compare the labeling patterns of control and halothane cultures.

Since the two-hour labeling period used to assess differences between leucine-³H incorporation in control and halothane cultures revealed no apparent differences we reasoned that a much longer labeling period might reveal that the inhibitory effect of halothane on growth and morphology was indeed the result of an interference with the protein synthetic machinery. Table 2 shows the results of a 48-hour labeling period. It is evident that even over this long labeling period there was no significant

TABLE 1. Incorporation of Leucine-³H into Cold TCA-precipitable Material by NB2a Cells*

Growth Conditions	Specific Activity (dpm/mg Protein)			
	30 Min	60 Min	90 Min	120 Min
Control				
Experiment 1	7170	14208	20957	32556
Experiment 2	13838	33680	47051	50939
Halothane				
Experiment 1	7161	13969	25135	25535
Experiment 2	20701	27951	37485	43991

* In experiment 1, cells were grown in control atmosphere (air-5 per cent CO₂) for 28 hours; half the dishes were placed in an incubator gassed with air-5 per cent CO₂-1 per cent halothane for 16 hours. Cells were labeled with leucine-³H at an isotope concentration of 3.2 μCi/ml and processed as described in Methods. For experiment 2, cells were grown in control atmosphere for 48 hours and half the dishes were placed in the 1 per cent halothane atmosphere for 21 hours; cultures were labeled at an isotope concentration of 5.9 μCi/ml. Each time point represents the mean specific activity of three dishes.

difference between halothane-grown and control cells.

A possible explanation of these data is that halothane exerts its effect by slowing the rate of protein degradation, *i.e.*, halothane may affect the turnover rate of protein in mouse neuroblastoma cells. To test this possibility, control and halothane cultures were labeled for four hours with leucine-³H. Triplicate cultures were assayed for TCA-precipitable radioactivity 4, 8, 24, 28 and 31 hours after labeling. The specific activities at each time point were compared by linear regression analysis, and the slopes of the two lines differed by 3 per cent or less. It is noteworthy that over the period of the long-term labeling and the turnover experiment, the growth rate of the experimental cells was approximately 50 per cent that of control cells. Since the data are expressed as specific activity (dpm/mg protein), the differences in protein are normalized and the labeling kinetics can be compared directly.

As the third approach to evaluating the effects of halothane on protein metabolism, we labeled cells grown in control or 1 per cent halothane atmospheres with ¹⁴C-amino acid hydrolysate for three hours. The cells were rinsed with saline solution twice and were directly solubilized with 1 per cent SDS and 1 per cent β-mercaptoethanol in 0.1 M phosphate buffer, pH 7.1. The samples were sonicated and heated to 100 C for 5 minutes. Samples were removed for protein determination and counting. They were then electrophoresed on 10 per cent polyacrylamide gels, stained with Coomassie blue, scanned, sliced and counted. Figure 4 shows the results of this experiment. Coomassie blue was used to take advantage of the sensitivity of the stain; as is seen, there was no detectable difference between the profiles of the control sample and the halothane sample.

When the patterns of radioactivity of the two samples are compared, once again no substantial difference is evident. Although there is no qualitative difference between these two gels, there is a possibility that quantitative differences could be found. For example, in the lower-molecular-weight region the control gel has a region of higher

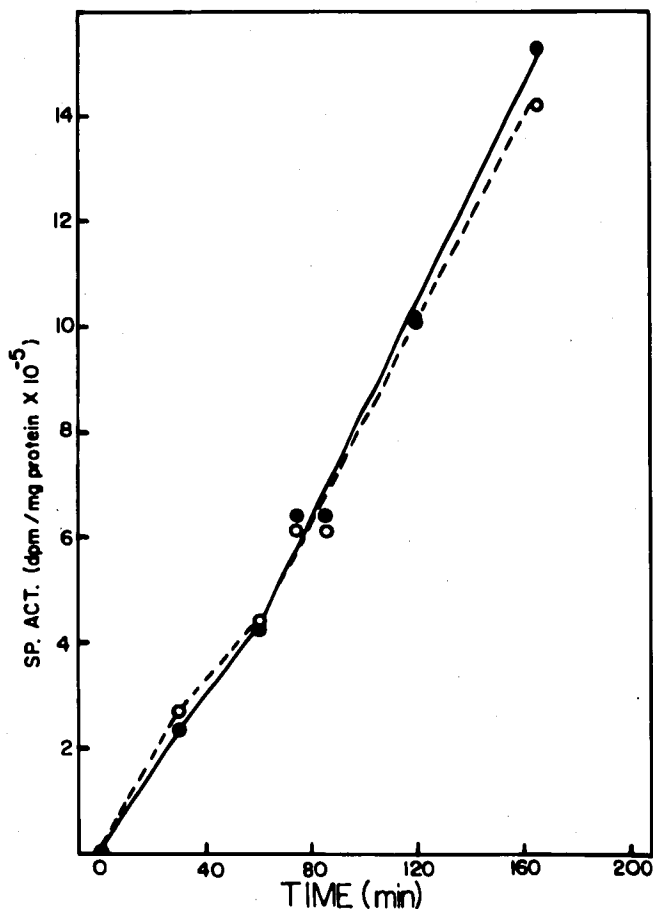


FIG. 3. Effect of 1 per cent halothane on leucine-³H incorporation into cold TCA-precipitable material. Each point represents the mean of several separate experiments; in every experiment each time point represents a duplicate or triplicate determination. See Methods for experimental details. Control —●—, halothane (1 per cent) ---○---

radioactivity than that in the halothane gel; however, such slight differences were inconsistent from one experiment to the next. The overall picture suggests similar, if not identical, patterns of protein synthesis and degradation in control and halothane-grown cells.

TABLE 2. Long-term Labeling of NB2a Cells by Leucine-³H*

Growth Conditions	Specific Activity (dpm/mg protein)				
	3 Hr	6 Hr	24 Hr	30 Hr	48 Hr
Control (air-5 per cent CO ₂)	30218 ± 10506	46970 ± 6485	118638 ± 8622	137584 ± 22266	139707 ± 17929
Experimental (air-5 per cent CO ₂ + 1 per cent halothane)	25605 ± 4585	43570 ± 6996	108597 ± 14748	119583 ± 20387	167202 ± 18805

* All cells were grown in control atmosphere for 24 hours and half the cultures were switched to halothane atmosphere for 16 hours. Each time point represents the mean and standard deviation for six cultures. The cells were labeled in 5 ml of medium at an isotope concentration of 2 μCi/ml.

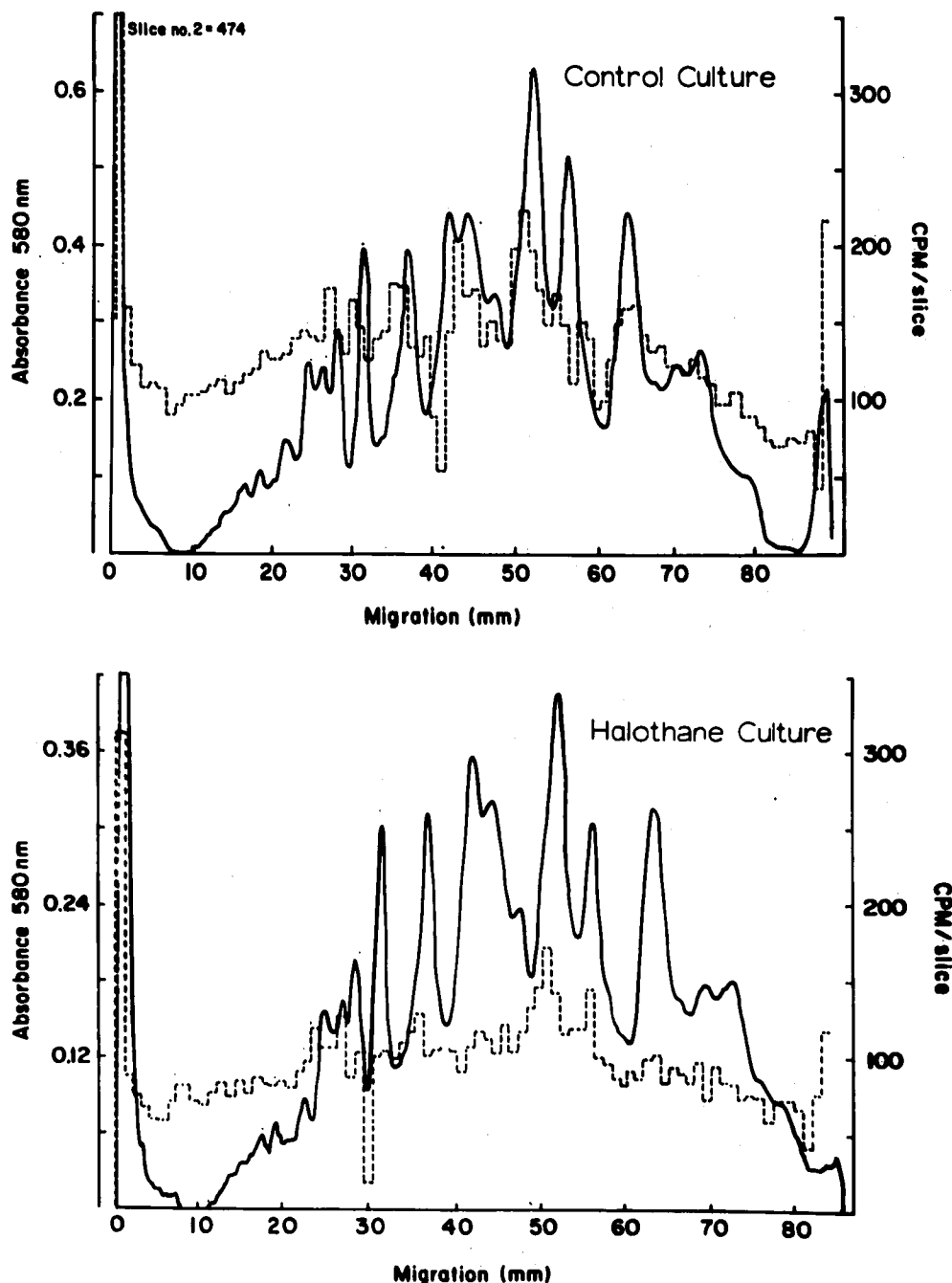


FIG. 4. SDS polyacrylamide gels of ^{14}C -amino acid-labeled protein from control (*above*) or 1 per cent halothane-grown (*below*) cultures. Dishes were inoculated with 2×10^5 cells and placed in a control incubator for 22 hours, then one dish was placed in an incubator gassed with 1 per cent halothane and both dishes were incubated for 19 hours. Both dishes were labeled for 3 hours in 3 ml of medium containing $2.5 \mu\text{Ci/ml}$ ^{14}C -amino acids. For running the gels, $25 \mu\text{l}$ of control cell solution ($65 \mu\text{g}$ protein, 6,000 cpm) and $35 \mu\text{l}$ of halothane-grown cells ($67 \mu\text{g}$ protein, 4,700 cpm) were applied to the gels.

In parallel cultures labeled with ^{14}C -amino acid hydrolysate, we measured the TCA-soluble and TCA-insoluble radioactivity. These data are presented in table 3.

INCORPORATION OF URIDINE- ^3H

The data in table 4 show that the incorporations of uridine- ^3H into total cold TCA-precipitable material did not differ significantly in control and halothane cultures. Figure 5 shows that when the mean specific activities (cpm uridine- ^3H per mg

protein) in control *vs.* halothane cultures from five separate experiments were plotted, there was no significant difference between the two cultures.

Further confirmation of these data was obtained by comparison of SDS-polyacrylamide gel electrophoresis patterns. These data are shown in figure 6. RNA was purified by cold phenol extraction of control and halothane-grown cells labeled with uridine- ^3H . The radioactive profiles showed that the patterns of labeling of RNA were essentially identical in the two cultures. From these radioactivity profiles, it is possible to calculate the ratio

of ribosomal RNA's synthesized in the control and halothane cultures. For the control cells, 44 per cent of the radioactivity was in the 28S and 18S regions of the gel; the ratio of radioactivity in the 28S/18S peaks was 2.3. For the halothane-exposed cells, 37 per cent of the radioactivity was in the 28S and 18S peaks, and the comparable ratio of 28S/18S was 1.9.

Discussion

The data presented in this paper demonstrate that mouse neuroblastoma cells grown in the presence of halothane show dose-dependent reductions in growth rate, ranging from about 30 per cent in 0.3 per cent halothane to more than 90 per cent in 1.5 per cent halothane.

On the basis of results from experiments in which we labeled protein or RNA with appropriate radioactive precursors, we conclude that the reduction in growth rate is not due to an inhibition of the rate of synthesis of these two major classes of macromolecules. Furthermore, a comparison of the radioactive patterns of proteins or RNA synthesized by the neuroblastoma cells by SDS-polyacrylamide gel electrophoresis revealed no apparent qualitative difference. Some minor quantitative differences were present, but these were not reproducible from experiment to experiment. We tried several methods to detect differences in protein synthesis and degradation in these cells. No difference was demonstrable by these different approaches. Examination of the TCA-soluble radioactivity in cells labeled with ¹⁴C-amino acids also showed that

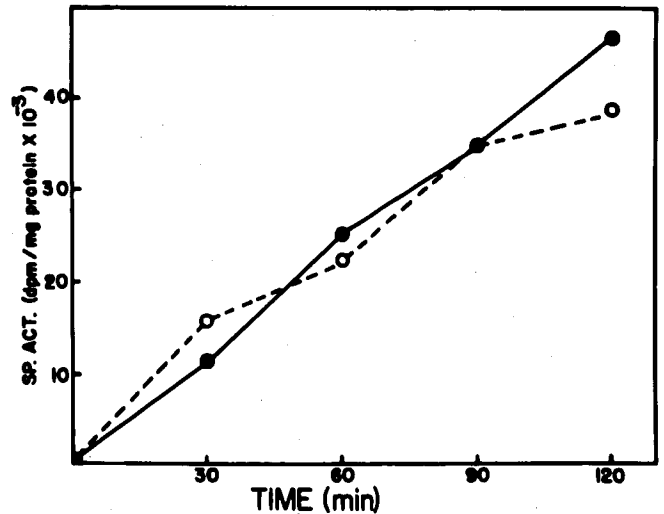


FIG. 5. Effect of 1 per cent halothane on the incorporation of uridine-³H into cold TCA-precipitable material in NB2a cells. Each point represents the mean value from five separate experiments. In each experiment, triplicate cultures were used for each time point. See Methods for the general protocol followed. Control —●—, halothane ---○---

halothane was not affecting the entry of these precursors into the cell.

Other reports on the effects of volatile anesthetics on macromolecular biosynthesis in mammalian cells have appeared. Ishii and Corbascio⁵ studied the effects of halothane on RNA, protein, DNA and lipid biosynthesis in minimal-deviation Morris hepatoma cells grown in spinner culture. Their results did

TABLE 3. Incorporation of ¹⁴C-labeled Amino Acids into Cold TCA-precipitable Material by NB2a Cells*

Growth Conditions	dpm Incorporated		dpm TCA-soluble dpm TCA-soluble + dpm TCA-insoluble	Specific Activity (dpm TCA-insoluble/ mg protein)
	TCA-insoluble	TCA-soluble		
Control (air-5 per cent CO ₂)	352858	107256	0.2331	159304
Experimental (air-5 per cent CO ₂ + 1 per cent halothane)	197006	57352	0.2255	137766

* All cells were grown in control atmosphere for 42 hours and then half the cultures placed in the halothane atmosphere for 40 hours. Triplicate cultures were labeled in 3 ml of medium containing 2.5 μCi/ml of ¹⁴C-labeled amino acids for two hours. The cells were scraped from the dishes with a rubber policeman, washed four times with phosphate-buffered saline solution (containing 5 μg/ml cycloheximide), sonicated, precipitated with TCA, and assayed for radioactivity as described in Methods.

TABLE 4. Incorporation of Uridine-³H into Cold TCA-precipitable Material by NB2a Cells*

Growth Conditions	Specific Activity (dpm/mg Protein)			
	30 Min	75 Min	120 Min	175 Min
Control (air-5 per cent CO ₂)	0.315 × 10 ⁶	0.637 × 10 ⁶	1.10 × 10 ⁶	1.54 × 10 ⁶
Experimental (air-5 per cent CO ₂ + 1 per cent halothane)	0.334 × 10 ⁶	0.615 × 10 ⁶	1.11 × 10 ⁶	1.43 × 10 ⁶

* All cells were grown in control atmosphere for 16 hours; half the cultures were then placed in the halothane atmosphere for 24 hours. Each time point represents the mean for four culture dishes. Cells were labeled in 2 ml of medium containing 6.25 μCi of uridine-³H per ml.

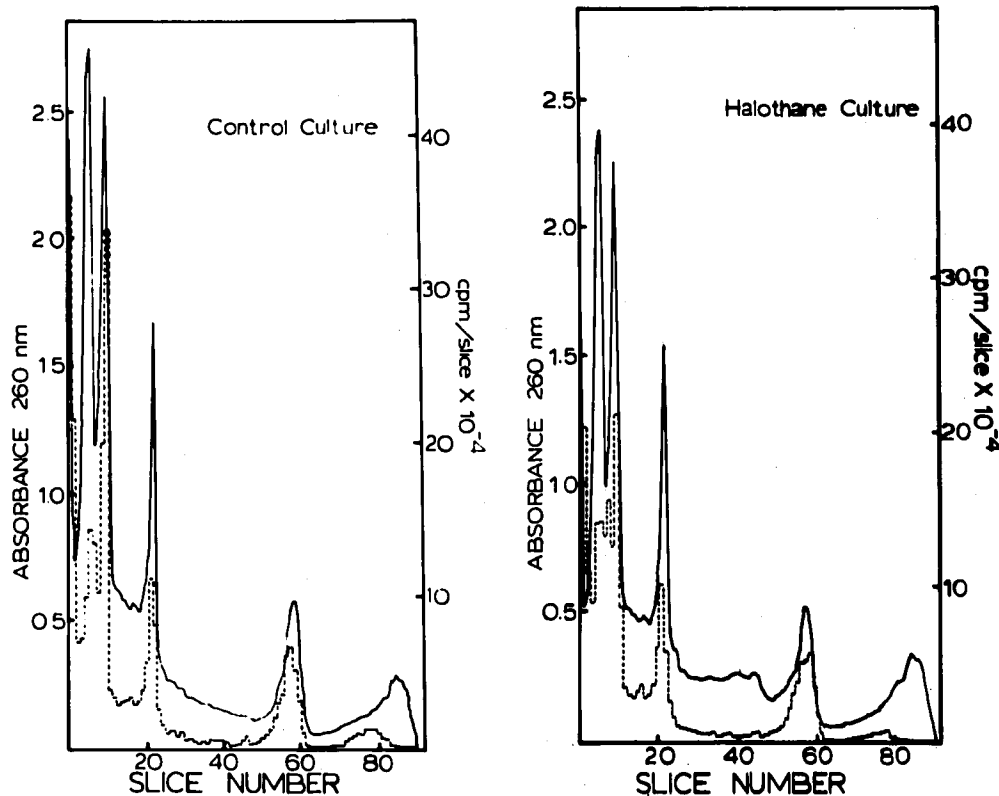


FIG. 6. SDS-polyacrylamide gel electrophoresis of uridine-³H labeled RNA from control (*left*) and 1 per cent halothane-grown (*right*) cells. Dishes containing 8×10^5 cells were grown for six hours in a control incubator and one dish was placed in a halothane-equilibrated incubator (1 per cent) for 18 hours. Cells were labeled for four hours in 5 ml of medium containing $5 \mu\text{Ci/ml}$ of uridine-³H. Cells were directly solubilized in 1 per cent SDS, 0.1 M acetate buffer, pH 5.0, containing $4 \mu\text{g/ml}$ polyvinyl-sulfate. Samples were applied to 2.7 per cent polyacrylamide gels (control $84 \mu\text{g}$ RNA, 2.35×10^6 cpm; halothane $74 \mu\text{g}$ RNA, 1.98×10^6 cpm).

not differ from ours except that they found an increase in lipid biosynthesis, a modality we did not investigate. Kramer and Poort⁷ found that diethyl ether and trichloroethylene inhibited protein synthesis in pancreatic exocrine cells of the rat. There are important differences between our studies and theirs that may explain the reported discrepancies: 1) they studied a tissue (the exocrine pancreas) that is actively engaged in secretory activity; 2) they anesthetized whole animals in their studies or incubated tissue slices *in vitro* in the presence and absence of anesthetics; 3) they did not use halothane as one of their test anesthetics. Others have reported that halothane does not inhibit protein synthesis in the liver, which is also an exocrine tissue.⁸ Therefore, we feel justified in concluding that halothane does not exert its effect on cells by interfering with the synthesis (or metabolism) of macromolecules.

Our results showing that halothane has no effect on the biosynthesis of protein and RNA provide further evidence that halothane does not exert its action on cells by interfering with the availability of energy (in the form of high-energy phosphate). RNA synthesis is particularly sensitive to the intracellular pools of ATP.²⁰ Early explanations of the action of anesthetics drew analogies between anesthesia and asphyxia, but recent data have shown that cerebral oxidative phosphorylation proceeds at the same rate as control rates (or slightly faster).²¹ A shift in energy production from oxidative to glycolytic pathways may occur,¹ but our data show that the net

effect on the cellular metabolism of macromolecules is not significant.

Several facts about 1 per cent halothane are pertinent to our conclusion. First, 1 per cent halothane in the gas phase is at the lower end of the concentration range used to achieve and maintain clinical anesthesia. Second, the growth rate of cultured mouse neuroblastoma cells at this concentration of halothane was inhibited approximately 50 per cent. Third, the intracellular organization of microfilaments 40–80 Å in diameter is completely abolished by 1 per cent halothane. Indeed, in our previous study¹³ we showed that even 0.3 per cent halothane causes complete but reversible disruption of the filaments in these cells. Furthermore, we and others^{9,22–24} have shown that these microfilaments bind heavy meromyosin and on this basis are identical to actin-like filaments that have been described to occur in a wide variety of cell types.²⁵

The dose-dependent inhibition of cell growth by halothane may be explained by a disruption of actin-like microfilaments in the mitotic apparatus. We have reported that in dividing mouse neuroblastoma cells there are filaments in the region of chromosomes that can be decorated with heavy-meromyosin, and that they appear to decrease in number following exposure to halothane.²⁶ Several other investigators^{27–30} have confirmed that there are actin-like filaments in the mitotic apparatus of mammalian cells.

Halothane apparently does not inhibit cell

division by disrupting spindle microtubules. We have no quantitative evidence to support this statement, but the following observations are consistent with it: When cells are cultured in the presence of halothane, the growth rate is reduced but growth does not cease. This reduction in growth rate is not a consequence of an increase in cell size in halothane-grown cultures. This is evident from the micrographs published in our earlier paper.¹³ Furthermore, cells do not appear to be blocked at metaphase, which appears to happen when a mitotic "poison" such as colchicine or vinblastine is added to growing cells. Spindle birefringence has been reported to be reduced in the presence of halothane.³¹ However, the microtubules of the mitotic apparatus appear morphologically normal (in both dimensions and number) in cells cultured in the presence of 1 per cent halothane.²⁶ If actin-like filaments have an important role in the functional integrity of the mitotic apparatus, as has recently been suggested,³² then the effects of halothane on cell morphology and growth rate in our experimental system may be solely due to interactions with actin-like microfilaments.

The interaction of halothane with proteins and lipoproteins has received considerable attention.³³⁻³⁵ From these studies it is clear that halothane can interact with hydrophobic regions in proteins and lipoproteins, causing a conformational change in the molecule. Such interactions could explain both enzymatic and structural effects of halothane. However, this type of mechanism lacks specificity, in that many proteins would be affected by halothane.

Twenty-five years ago it was shown that volatile anesthetics exerted a differential effect on axons and synapses.³⁶ More recently, Nicholl showed that at very low halothane concentrations (0.6 mM) nerve impulse conduction is unaffected, while synaptic transmission is virtually blocked.³⁷ The presence of actin-like proteins in the central nervous system is well documented.³⁸⁻⁴⁰ Concentrations of actin-like filaments have been demonstrated at the cytoplasmic surfaces of presynaptic plasma membranes.⁴¹ The possibility that halothane inhibits the transmission of nerve impulses at synaptic endings by disrupting a system of synaptic microfilaments that are necessary for normal function must be considered.

Studies of the interaction of halothane with muscle proteins have appeared. Halothane interacts with skeletal muscle myosin and the K⁺-activated (but not the Ca⁺⁺-activated) ATPase of the same myosin.⁴² Price *et al.*⁴³ have reported indirect evidence that halothane interferes with the troponin-troponin system of cardiac muscle. Direct studies on the effect of halothane on the F-actin \rightleftharpoons G-actin transformation or on the ATPase activity of actomyosin complexes may provide conclusive evidence for a specific effect of halothane on the

subplasmalemmal complex of actin and myosin-like proteins recently described to occur in so many non-muscle cells.

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