ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1980, p. 890-896
0066-4804/80/05-0890/07$02.00/0
Vol. 17, No. 5

Anesthetic Action and Virus Replication: Inhibition of Measles Virus Replication in Cells Exposed to Halothane

PAUL R. KNIGHT,[1,2] MICHAEL L. NAHRWOLD,[1] AND ELLIOTT BEDOWS[1,2]

Departments of Anesthesiology[1] and Epidemiology,[2] University of Michigan, Ann Arbor, Michigan 48109

Replication of measles virus in BSC cells was studied in the presence of halothane, a commonly used volatile anesthetic. At clinical concentrations of the anesthetic, appearance of progeny virus was decreased in a dose-related manner. This inhibition was reversible as the removal of halothane allowed virus replication to be resumed. Studies attempting to elucidate the mechanism of action of the anesthetic inhibition of virus replication revealed that halothane did not directly inactivate the virus particle or prevent viral adsorption to the cell. Infectious virus and nucleocapsid production were decreased or stopped, depending on the anesthetic dosage used. Direct immunofluorescent staining for measles virus antigen was negative in cells treated at the higher concentrations of halothane. Recovery of nucleocapsid production started within a few hours after removal of halothane. Furthermore, the combined inhibitory effects on viral ribonucleic acid synthesis of 5-azacytidine and halothane were additive. This evidence suggests that inhibition of measles virus replication occurs at or before ribonucleic acid synthesis.

Halothane (2, bromo-2-chloro-1,1,1, trifluoroethane) is the most commonly used inhalational anesthetic vapor clinically in use in the United States today. However, there is a paucity of information regarding the morbidity and mortality associated with the use of this or any other anesthetic agent in patients harboring viral agents. Halothane exposure is reported to increase mortality in mice infected with mouse hepatitis virus (12). Diethyl ether exposure, however, decreases the severity of certain viral illnesses of mice (15, 16) and canines (2, 3). The pathogenesis of still other viral diseases remains unchanged in mice exposed to ether (15, 16).

Although several investigators (1, 7, 8, 17) have studied macromolecular synthesis in cells exposed to anesthetics, there are no available data pertaining to synthesis of virus components in these cells. At high doses, halothane inhibits cellular deoxyribonucleic acid (DNA) and protein synthesis. Variable effects of the anesthetic on ribonucleic acid (RNA) synthesis have also been reported (1, 7, 17). These observations suggest that halothane may alter virus replication.

The present investigation attempts to examine the perturbations produced by halothane on cellular and measles virus replication, and virus macromolecular synthesis. Measles virus is closely related to distemper virus, which causes a neurological disease in canines that can be attenuated by exposure of the animal to the anesthetic diethyl ether (2, 3). The following report describes the replication of measles virus in halothane-treated cells in tissue culture. We also examine production of measles virus components intracellularly in the presence of this anesthetic.

(This study was presented in part at the Annual Meeting of the American Society of Anesthesiologists, Chicago, Ill., October 1978.)

MATERIALS AND METHODS

Virus. Stocks of CC measles virus, whose history has been described (6), were grown in BSC cells. After 90% of the cells in infected cultures demonstrated measles virus-specific cytopathic effect (CPE), virus samples were harvested by sonic disruption of the cells. Virus stocks were quick frozen in a dry ice-alcohol bath and stored at −70°C.

Cells. BSC cells, provided by F. Rapp of the Microbiology Department of the Hershey Medical Center, were originally obtained from R. Dulbecco at the Salk Institute.

Stock cell lines were grown in 8-ounce (ca. 241 ml) prescription bottles. All cells were grown in Eagle basal medium supplemented with 10% fetal calf serum, 10% tryptose phosphate broth, 0.08% $NaHCO_3$, and antibiotics. Changes in this basic formula were made as noted below.

Plaque assay. The plaque assay was utilized to determine infectious measles virus titers. Briefly, BSC cells grown in 60-mm^2 plastic petri dishes were inoculated with 0.4 ml of the measles virus suspension to be assayed. After virus adsorption for 2 h at room temperature in the dark, the BSC cell monolayers were overlaid with Eagle basal medium containing 10% fetal

calf serum, 1% agar, 0.23% $NaHCO_3$, and antibiotics. Infected cultures were kept in 95% air–5% CO_2 at 37°C for 4 days, after which time 2 ml of tris(hydroxymethyl)aminomethane)(Tris)-buffered saline (pH 7.4, 0.025 M) containing a 1:20,000 dilution of neutral red was added. Plaques were counted 5 days after initial virus adsorption. Each assay was performed in triplicate.

Exposure of cells to halothane. Cell cultures were grown to confluency in sealed 8-ounce prescription glass bottles containing 10 ml of media. Plastic caps fitted with silicone rubber septa coated with Teflon were used to seal the flasks. Halothane vapor was provided by directing a 95% air–5% CO_2 mixture at 5 liters/min through a Dräger vaporizer. The vaporizer output was connected to a 20-gauge needle placed in the septum; a second 20-gauge needle provided an outlet for the flask. Each bottle was equilibrated for 20 min, and halothane concentrations were assessed by gas chromatography. Preliminary experiments demonstrated that the culture medium was equilibrated with halothane vapor within 10 min. Halothane levels were checked routinely every 24 h, and the bottles were re-equilibrated by exposure for 10 min to halothane as described above. The final concentration of halothane in the sealed culture flasks after 24 h was within 5% of the initial concentration. For studies of the reversibility of halothane's effect, the cell monolayers were washed two times with fresh media and exposed to a stream of 95% air–5% CO_2 for 15 min. Cultures were then washed again with fresh media. With this method, residual halothane concentrations were less than 0.02% in all cultures as determined by gas chromotography.

Virus adsorption assays. Monolayers of BSC cells in 8-ounce bottles were inoculated with 100 plaque-forming units (PFU; 1 ml) of measles virus in the presence or absence of 1.8% halothane. After incubation at 37°C for various times, the anesthetic was removed. The monolayers were washed three times with Tris-buffered saline and overlaid with agar. The amount of cell-associated virus (adsorbed virus) was then determined by the number of plaques appearing in the culture.

In a similar experiment 8-ounce (ca. 241-ml) bottles of confluent BSC cells were inoculated with 10^6 PFU (1 ml) of measles virus. At various times the amount of virus in the medium (unadsorbed virus) was assayed by the plaque assay.

Immunofluorescence technique. Cells were washed three times in warm (37°C) Tris-buffered saline (pH 7.4), air dried, and fixed in acetone for 5 min. Fluorescein isothiocyanate-labeled anti-measles virus gamma globulin prepared against whole virus was adsorbed to the cover slips for 30 min at 37°C. After the cover slips were washed three times in warm Tris-buffered saline (pH 7.4), they were mounted and viewed with a Zeiss fluorescence microscope.

Viral RNA synthesis in halothane-treated cellls. To assess the effect of halothane on viral RNA replication, we exposed measles virus-infected cells to 25 μg of 5-azacytidine per ml, an inhibitor of RNA replication, after cultures had been exposed to halothane for 24 h. The halothane was removed immediately after the addition of the inhibitor. Progeny virus were assayed by the plaque technique 48 h postinfection.

Isolation of measles virus nucleocapsids. Measles virus nucleocapsids were isolated essentially by the method of Kiley et al. (9). Briefly, BSC cells were grown to confluency in 8-ounce prescription glass bottles. Cultures were exposed to 95% air–5% CO_2 with or without halothane. Virus was inoculated (0.05 PFU/cell) in these cultures and allowed to adsorb for 1 h. Twenty-four hours postinfection, 20 μg of actinomycin D (Sigma Corp.) per ml was added, and 1 h later 20 μCi of [5-^{3}H]uridine (New England Nuclear Corp.; 30 Ci/mmol) label per ml was added. At 40 h after infection, cells were harvested. A cytoplasmic extract was prepared as follows. The cells were pelleted at 800 × *g* for 10 min, washed once in Tris-buffered saline, and suspended in 1 to 3 ml of hypotonic buffer. Cells were disrupted by 15 strokes of a Dounce homogenizer. Nuclei were pelleted at 1,500 × *g* for 5 minutes. Labeled structures in the decanted cytoplasmic extracts were separated by rate-zonal centrifugation, and radioactivity incorporation into RNA was determined by liquid scintillation counting.

Host cell protein and RNA synthesis. To determine whether experimental doses of halothane were toxic to the protein-synthesizing apparatus of the cell, the following assay was performed. BSC-1 cells were grown in 8-ounce prescription bottles. Half of these cultures received halothane as previously described. Two hours after exposure to halothane, 100 μCi of L-[5^3-H]isoleucine (Schwartz/Mann) was added to both cultures which had or had not received the anesthetic. After an incubation of 4 h at 37°C, the cells were harvested, washed three times in 150 mM NaCl containing 40 mM Tris buffer (pH 7.8), frozen, and thawed, and acid-precipitable radioactive material was collected on a 0.45-μm nitrocellulose filter (Schleicher and Schuell) after precipitation in 10% trichloroacetic acid. Total protein was determined by the method of Lowry et al. (11).

Host cell RNA synthesis was determined in a similar manner with 20 μCi of [5-^{3}H]uridine (New England Nuclear Corp.) per bottle except that lysed cells were clarified at 10,000 × *g* for 10 min before supernatant fractions were assessed for acid-precipitable counts.

RESULTS

Toxicity of halothane to cells and virus. Halothane at the highest concentration studied in these experiments, 2.5%, did not appear to produce irreversible toxicity to the cells. Light microscopy demonstrated that halothane-treated cells were more spherical and refractile to the light than the unexposed cells. These changes disappeared within 1 h after removal of the anesthetic. Despite the change in morphology, there was no change in cell viability determined by using the trypan blue dye exclusion technique. Cells exposed to halothane concentrations greater than 1.5% did not replicate until removal of the anesthetic as assayed by daily cell counts. However, at halothane concentrations as high as 2.1%, the incorporation of L-[5-

^{3}H]isoleucine into host cell protein was only minimally decreased, whereas [5-^{3}H]uridine uptake into host cell RNA was not significantly affected (data not shown).

The rate of thermal inactivation of measles virus at 37°C in the presence or absence of halothane was followed for 24 h. These data (Fig. 1) demonstrated the lack of an increase in thermal inactivation of measles virus infectivity when the virus was exposed to anesthetic concentrations of 0 to 2.5%.

Replication of measles virus in halothane-treated cells. Figure 2 depicts the production of infectious measles virus in the absence and presence of halothane at different doses. It is clearly seen that halothane dramatically reduced infectious virus titers. Growth patterns were similar in cultures to which the anesthetic had been added as late as 12 h after adsorption. These growth curves are characterized by two features: a delay in the onset of virus multiplication and a reduction in virus yield at the time of peak titer (48 h postinfection). The maximum yields of infectivity decreased progressively with increasing concentrations of anesthetic. At anesthetic concentrations of 1.5 to 1.8% halothane, measles virus did not replicate at all.

A corresponding inhibitory effect of the anesthetic on the development of virus-specific CPE

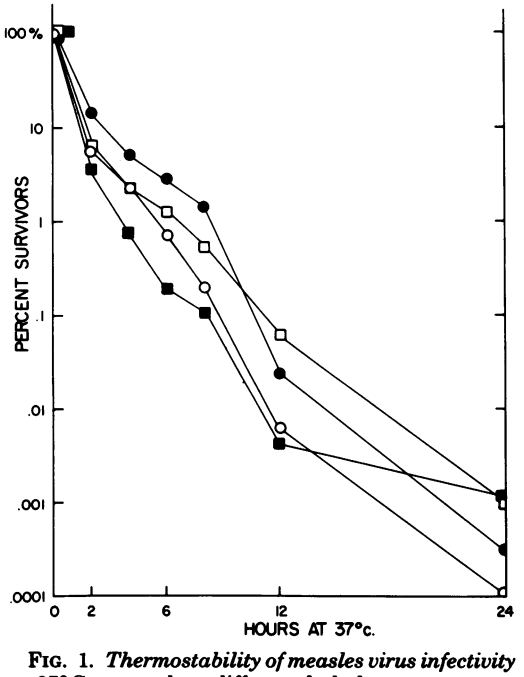

FIG. 1. *Thermostability of measles virus infectivity at 37°C exposed to different halothane concentrations:* ○, *0%;* ●, *0.8%;* ■, *1.5%;* □, *2.5%.*

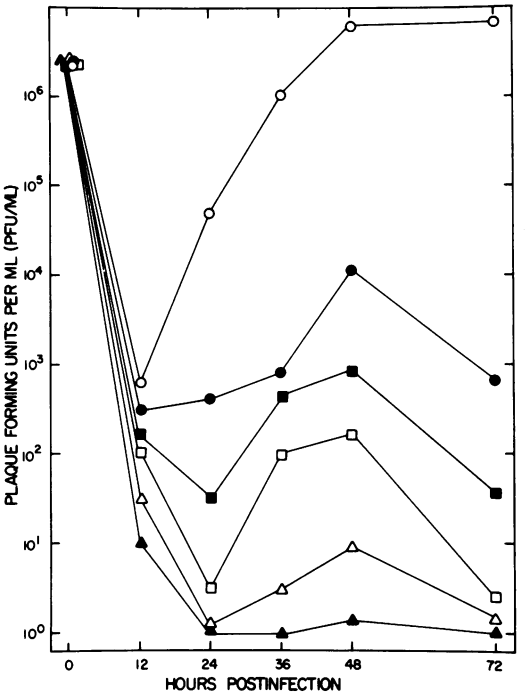

FIG. 2. *Comparative replication of measles virus after exposure of cultures to different halothane concentrations:* ○, *0%;* ●, *0.8%;* ■, *1.0%;* □, *1.2%;* △, *1.4%;* ▲, *1.5%.*

was found. In cultures treated with 1.8% halothane there was no CPE at 48 h postinfection, whereas cells not exposed to the anesthetic demonstrated 100% CPE. The amount of CPE seen at different anesthetic doses was a gradation between these two extremes.

Effect of halothane on adsorption of measles virus to BSC cells. To determine whether halothane exerts an effect on adsorption of measles virus to BSC cells, we exposed monolayer cultures to virus at 37°C in the presence and absence of halothane. Two experiments were performed. In one we assayed adsorbed virus, and in the other we assayed unadsorbed virus. Table 1 shows that halothane has no effect on adsorption of measles virus to BSC cells as a function of time as determined by either the adsorbed or unadsorbed virus techniques.

Reversibility of antiviral activity of halothane by removal of the anesthetics. The reversibility of the antiviral activity of halothane was examined. Figure 3 shows the production of infectious measles virus in cells exposed to 1.8% halothane immediately after virus adsorption and when halothane was removed at various times postadsorption. Replication of measles virus was not affected by the anesthetic if removal

TABLE 1. *Adsorption of measles virus to BSC cells in the presence or absence of halothane*[a]

Halothane concn (%)	Incubation period (min)	Unadsorbed virus[b] (PFU/ml)	Adsorbed virus[c] (PFU/ml)
0	0	1.3×10^6	–
	5	9.5×10^5	55
	10	8.7×10^5	67
	20	6.2×10^5	71
	30	5.2×10^5	82
	45	2.1×10^5	85
1.8	0	1.1×10^6	–
	5	9.2×10^5	54
	10	7.6×10^5	64
	20	6.1×10^5	69
	30	4.3×10^5	87
	45	1.9×10^5	83

[a] Cultures of BSC cells were incubated at 37°C with 2×10^6 PFU (experiment 1) or 100 PFU (experiment 2) in the presence or absence of 1.8% halothane. At indicated times the medium was harvested and assayed for infectious virus from replicate cultures, one of which was exposed to halothane. In experiment 2, replicate cultures, one containing halothane, were washed twice with Tris-buffered saline, and the anesthetic was removed from the exposed culture.

[b] Experiment 1. Unadsorbed virus was assayed for infectivity by the plaque technique.

[c] Experiment 2. After the cultures were washed, they were overlaid with agar, and the cell-associated virus (adsorbed virus) was determined by the number of plaques appearing in the culture.

occurred before 12 h postinfection. On the other hand, if the anesthetic was not removed until after 12 h a delay in the appearance of progeny virus occurred and recovery of peak titers was delayed. Peak titers did, however, eventually approach control levels after removal of the anesthetic, suggesting reversal of the anesthetic antiviral effects.

Synthesis of measles virus nucleocapsids in the presence of halothane. During normal paramyxovirus replication, protein-encapsidated genomic RNAs (nucleocapsids) are synthesized and then processed into mature, infectious viral particles. The amount of nucleocapsid material recovered from an infected cell lysate is not only many times greater than the number of infectious viral particles but also represents an early stage in the viral assembly. Thus, measuring the synthesis of nucleocapsid material provides a sensitive assay for the synthesis of virus-specific products, whose subsequent processing may be inhibited.

Measles virus nucleocapsid synthesis was examined in the presence or absence of halothane. Figure 4A shows the effect of different anesthetic doses on nucleocapsid production. The inhibition of this viral component corresponded well to the reduction in infectivity produced by the anesthetic. At 1.8% halothane completely inhibited the incorporation of tritiated uridine into nucleocapsid material. Removal of the anesthetic at 33 h postadsorption resulted in recovery of the appearance of nucleocapsids (Fig. 4B). Their appearance 3 h after the removal of halothane foreshadowed the recovery of virus infectivity (infectivity peaks 12 to 14 h after anesthetic removal). It is of interest that the nucleocapsids produced in the presence of lower doses of halothane and those which appeared after the removal of the anesthetic had a predominance of slowly sedimenting types by sucrose centrifugation.

Effect of halothane on virus protein and RNA synthesis. To further investigate the possible site(s) of halothane-induced biochemical inhibition of measles virus replication, we chose to look at the effect of the anesthetic on virus protein and RNA synthesis. To determine whether virus-specific protein was synthesized, we treated cells with measles virus-specific immunofluorescent antiserum, and positive-stain-

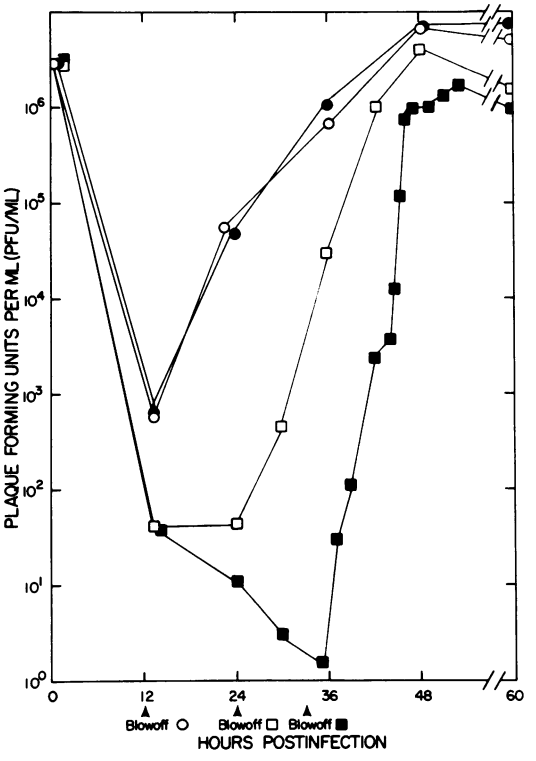

FIG. 3. *Comparative replication of measles virus exposed to 1.8% halothane, demonstrating the reversibility of the anesthetic. The halothane vapor was removed (blowoff) at (hours postinfection): ○, 0; ●, 12; □, 24; ■, 33.*

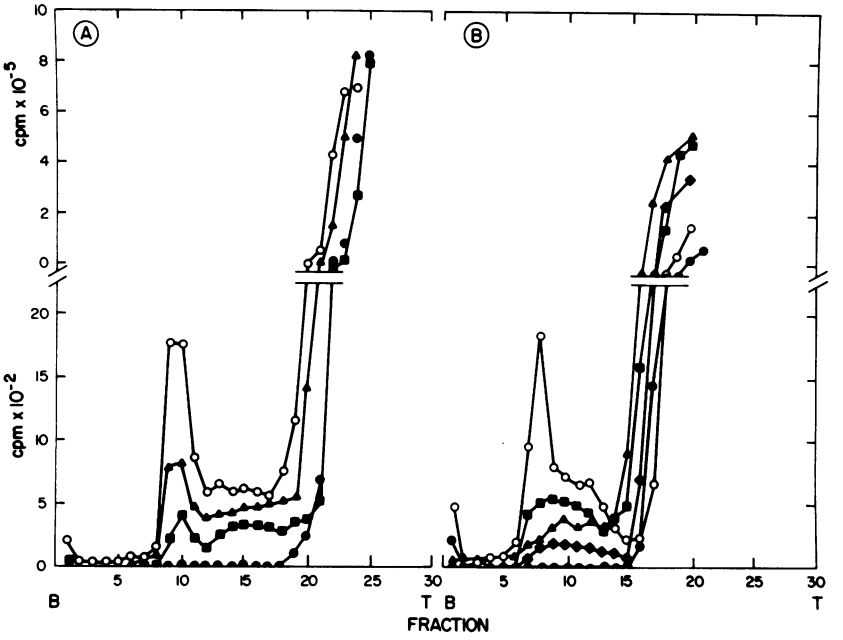

FIG. 4. *Effect of halothane on measles virus nucleocapsid production. Measles virus nucleocapsids were prepared as described in the text with the bottom of the gradient to the left. (A) The amount of nucleocapsid material synthesized in BSC cells in the presence of 0%* (○), *0.9%* (▲), *1.4%* (■), *or 1.8%* (●) *halothane. (B) The amount of nucleocapsid material synthesized after removal of 1.8% halothane. Infected BSC cells were exposed to halothane for 33 h. Cultures were harvested at 0 h* (●), 3 h (◆), *8 h* (▲), *or 14 h* (■) *after the removal of halothane, and compared to a control culture* (○) *which had never been exposed to the anesthetic.*

ing cells were determined. Infected BSC monolayers not exposed to halothane showed measles virus antigen-specific staining in 80 to 90% of the cells at 36 h after adsorption (Fig. 5A). However, in the presence of 1.8% halothane, the measles virus-infected cells did not stain positively for measles virus antigen (Fig. 5B). This indicates that these immunofluorescent specific viral proteins were not being synthesized or were antigenically altered during exposure to halothane.

To further determine whether RNA was being synthesized, we investigated what effect the combined action of halothane and 5-azacytidine had on infectious virus production. 5-Azacytidine has been shown to mutagenize and inactivate RNA viruses (5). Studies have shown that if the analog is added after measles RNA synthesis is initiated, inhibition of infectious virus production is not complete (10). However, when 5-azacytidine is added immediately after adsorption of measles virus, infectious progeny virus is not produced. Thus, if we treat the virus-infected cells with an anesthetic agent which inhibits viral RNA synthesis and, after the removal of this agent, add 5-azacytidine, infectious progeny virus would not be expected to appear.

In this experiment measles virus-infected cells were initially grown in the presence or absence of 1.8% halothane for the first 24 h after infection. After 24 h the anesthetic was removed, and 5-azacytidine was added to both cultures. Table 2 shows that when 5-azacytidine was added to cells without halothane treatment 24 h after adsorption, the virus yield was reduced slightly to 26% of control cultures which never received the analog. However, when cells were treated with halothane for 24 h before the addition of 5-azacytidine, the yield of infectious virus was very low, suggesting that the viral RNA synthesis had been inhibited by halothane.

DISCUSSION

Our results reveal that halothane exposure inhibits the production of infectious measles virus in BSC cells. The halothane concentrations used in this study were similar to those used clinically. The inhibition of virus replication is dose-related, ranging from a slight decrease in virus titer to complete inhibition of progeny virus production. In addition, the cell fusion type of the CPE characteristic of measles virus infections was not observed at halothane concentrations which inhibited virus production. Inhibi-

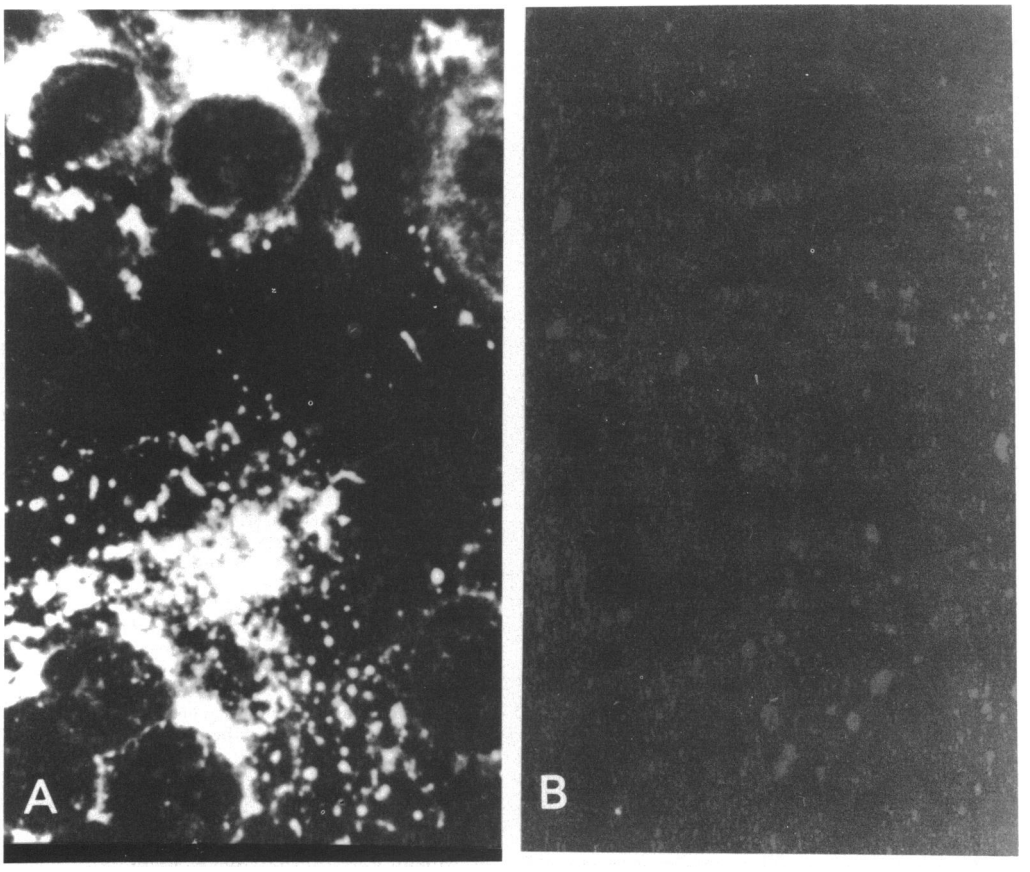

FIG. 5. *(A) Immunofluorescence photomicrograph of measles virus-specific antigen in the cytoplasm of BSC cells infected with measles virus for 36 h (×360). (B) Immunofluorescence photomicrograph of BSC cells infected with measles virus and treated with 1.8% halothane for 36 h. Note the lack of staining of virus-specific antigen in the cytoplasm of the cell (×360).*

TABLE 2. *Effect of 5-azacytidine on measles virus replication after treatment with halothane for 24 h*[a]

Halothane concn (%)	5-azacytidine concn (μg/ml)	Infectious virus yield (PFU/ml) (% of control)
(A) 0	0	2.7×10^6 (100)
(B) 0	25	7.0×10^5 (28)
(C) 1.8	0	1.3×10^6 (48)
1.8	25	2.0×10^3 (0.07)

[a] BSC cells were infected with 2×10^6 PFU of measles virus per ml and exposed to halothane (1.8%) for 24 h. Halothane was then removed and 25 μg of 5-azacytidine per ml was added. These results were compared to controls receiving (A) no treatment, (B) 25 μg of 5-azacytidine per ml 24 h postinfection, or (C) 1.8% halothane for the first 24 h. Cultures were harvested 48 h postinfection and assayed for infectious virus by the plaque technique.

tion of measles virus replication is reversible when halothane is removed from the system.

We examined the mechanism by which halothane inhibits measles virus replication. Adsorption of virus to cells was not prevented by halothane. This is interesting because this interaction involves lipid membranes, a presumed site of anesthetic action. However, halothane treatment of infected BSC cells does appear to alter virus macromolecular synthesis. Viral nucleocapsids could not be detected at inhibiting doses of halothane. Consistent with this finding was the observation that cytoplasmic measles virus-specific antigen as detected by immunofluorescence staining could not be found in infected cells exposed to halothane. In addition, inhibition of virus replication by 5-azacytidine, an RNA inhibitor, was additive in halothane-treated cultures. Thus, synthesis of virus protein

and RNA in these experiments appears to be affected. These observations could result from halothane action at an early step of virus replication after adsorption, but at or before specific virus RNA synthesis. Halothane has been shown to adversely affect cellular microtubule assembly (13), and the preponderance of slowly sedimenting nucleocapsids at low halothane doses and after the removal of halothane may relate to this observation.

Other work on halothane-induced alterations of cellular RNA synthesis has shown a discrepancy in results. Bruce, using phytohemagglutinin-treated human lymphocytes, found that 2% halothane caused up to 50% reduction of RNA synthesis and somewhat less inhibition of protein synthesis (1). Other investigators have not reported similar results (7, 17). One group demonstrated that RNA synthesis in neuroblastoma cells is not altered as assayed by either total RNA synthesis or by the incorporation of radioactive label into different RNA species (17). Protein synthesis was only minimally decreased in this study. Our results on host cell protein and RNA synthesis are consistent with these latter findings.

Ether anesthesia increases survival of dogs infected with distemper virus (2, 3) and mice infected with western and eastern equine encephalitis and St. Louis encephalitis viruses (15, 16). The mechanism of this protection is not likely due to a direct effect on the virion because the concentrations of ether the mice received were 15 times less than the concentration which has direct virucidal activity. Diethyl ether inhibition of the pathogenesis of these diseases may occur by a mechanism similar to the one we observed here with halothane on measles virus replication.

Anesthetics do not always cause a favorable outcome in virus-host interactions. The diseases caused by polio and rabies virus appear resistant to ether (15, 16), and halothane causes an exacerbation of the disease occurring in mice infected with mouse hepatitis virus (12). In humans, the combination of anesthesia and surgery causes acute viral hepatitis to be more severe, although chronic hepatitis does not seem to be affected (4). There is also an increased morbidity associated with upper respiratory tract infections during anesthesia and surgery (14).

We have demonstrated a depressant effect of an inhalation anesthetic, halothane at clinical concentrations, on a human viral agent. Many patients undergoing anesthesia and operating-room personnel undergoing chronic exposure to anesthetic agents may harbor endogenous viruses; therefore, further studies of anesthetic effects on the host-virus relationships should be pursued.

ACKNOWLEDGMENTS

We thank Francis E. Payne and Peter J. Cohen for their criticism during the preparation of this manuscript. In addition, we thank Jean Gaymer, Bruce Davidson, and Kim Bona for their technical assistance.

LITERATURE CITED

1. **Bruce, D. L.** 1975. Halothane inhibition of RNA and protein synthesis of PHA-treated human lymphocytes. Anesthesiology **42**:11–14.
2. **Donovan, C. A.** 1967. The use of diethyl ether inhalation in canine distemper. Vet. Med. Small Anim. Clin. **62**: 658–660.
3. **Donovan, C. A.** 1968. The influence of diethyl ether inhalation on experimental canine distemper. Vet. Med. Small Anim. Clin. **63**:345–347.
4. **Dykes, M. H. M.** 1970. Anesthesia and the liver, vol. 8, p. 71–87. Little, Brown & Co., Boston.
5. **Halle, S.** 1968. 5-Azacytidine as a mutagen for arboviruses. J. Virol. **2**:1228–1229.
6. **Haspel, M. V., P. R. Knight, R. Duff, and F. Rapp.** 1973. Activation of a latent measles virus infection in hamster cells. J. Virol. **12**:690–695.
7. **Ishii, D. N., and A. N. Corbascio.** 1971. Some metabolic effects of halothane on mammalian tissue culture cells in vitro. Anesthesiology **34**:427–438.
8. **Jackson, S. H. and F. Rapp.** 1973. The metabolic effects of halothane on mammalian hepatoma cells in vitro. II. Inhibition of DNA synthesis. Anesthesiology **39**:405–409.
9. **Kiley, M. P., R. H. Gray, and F. E. Payne.** 1974. Replication of measles virus: distinct species of short nucleocapsids in cytoplasmic extracts of infected cells. J. Virol. **13**:721–728.
10. **Knight, P. R., R. Duff, and R. Rapp.** 1972. Latency of human measles virus in hamster cells. J. Virol. **10**:995–1001.
11. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
12. **Moudgil, G. C.** 1973. Influence of halothane on mortality from murine hepatitis virus (MHV_3). Br. J. Anaesth. **45**:1236.
13. **Nunn, J. F., and A. C. Allison.** 1972. Effects of anesthetics on microtubular systems, p. 138–148. *In* B. R. Fink (ed.), Cellular biology and toxicity of anesthetics. The Williams & Wilkins Co., Baltimore.
14. **Rovenstine, E. A., and I. B. Taylor.** 1936. Postoperative respiratory complications: occurrence following 7874 anesthesias. Am. J. Med. Sci. **191**:807–819.
15. **Sulkin, S. E., and C. Zarafonetis.** 1947. Influence of anesthesia on experimental neurotropic virus infections. II. In vitro studies with the viruses of western and eastern equine encephalomyelitis, St. Louis encephalitis, poliomyelitis (Lansing), and rabies. J. Exp. Med. **85**: 559–569.
16. **Sulkin, S. E., C. Zarafonetis, and A. Goth.** 1946. Influence of anesthesia on experimental neurotropic virus infections. I. In vivo studies with the viruses of western and eastern equine encephalomyelitis, St. Louis encephalitis, poliomyelitis (Lansing), and rabies. J. Exp. Med. **84**:277–292.
17. **Telser, A., and R. E. Hinkley.** 1977. Cultured neuroblastoma cells and halothane: effects on cell growth and macromolecular synthesis. Anesthesiology **46**:102–110.