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Effect of Halothane on the Replication of Animal Viruses

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Five RNA- and two DNA-containing viruses were propagated in Vero cells and tested for their ability to replicate in the presence of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), a commonly used inhalational anesthetic. Halothane did not affect poliovirus replication at any anesthetic concentration tested, but all other viruses were either partially or totally inhibited by clinical doses of the anesthetic. Replication of Sendai virus, simian virus 40, vesicular stomatitis virus, and herpes simplex virus type 1 were moderately inhibited by halothane exposure. At concentrations of 2.2% (vol/vol) halothane, peak virus titers were reduced by ca. 2 orders of magnitude for vesicular stomatitis virus and simian virus 40, 3.5 orders of magnitude for Sendai virus, and 4 orders of magnitude for herpes simplex virus. Newcastle disease virus and measles virus were the most susceptible to exposure to halothane. Total inhibition of the replication of these viruses occurred at 1.6 to 2.0% halothane. All of the viruses whose replication was susceptible to the action of halothane were inhibited in a concentration-dependent manner. Furthermore, with the exception of simian virus 40, the inhibition of the replication of all viruses was reversible after halothane removal, although total recovery of virus synthesis was not observed unless the culture medium was changed or the pH was adjusted after anesthetic removal.

Volatile, general inhalational anesthetics possess antiviral properties. Exposure of measles virus (MV)-infected BSC-1 (monkey kidney) cells in culture to halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) results in a decrease in infectious progeny virus (11). Enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether) and its chemical isomer isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) also inhibit MV replication in a concentration-dependent and reversible manner in BSC-1 cells (12). All three anesthetics produce identical decreases in peak MV titers at clinically equipotent concentrations. However, recovery of MV replication after removal of anesthetic vapors from exposed BSC-1 cells is different with each anesthetic. Virus replication after removal of isoflurane in MV-infected BSC-1 cells is slow and never totally recovers, whereas it is rapid and complete with either halothane or enflurane. However, an increase in the synthesis of MV nucleocapsids with less than full-length RNA is observed after removal of halothane from either infected BSC-1 or Vero cultures. This increase in short nucleocapsids is not observed in BSC-1 cells after enflurane removal, and only a very slight increase in the amount of slowly sedimenting particles occurs after isoflurane removal (12).

In an attempt to understand the mechanism whereby virus replication is inhibited by inhalational anesthetics, we have studied the effects that one of these agents, halothane, has on two DNA-containing viruses which assemble in the nucleus of the host and five RNA-containing viruses which assemble at various sites within the cytoplasm, all of which could be propagated in Vero cells. The results, which shed light on the mechanism of action of general anesthetics, are described below.

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MATERIALS AND METHODS

Cells. Vero cells were grown in Eagle minimum essential medium (MEM) containing 10% fetal bovine serum, 100 μg of penicillin per ml, and 100 μg of streptomycin per ml as described previously (2). Cells were passaged with 1:8 dilutions weekly.

Viruses. All viruses used in these experiments were propagated for at least three passages in Vero cells before use in the experiments described below. The growth of the wild Edmonston strain of MV (2) and of the KOS strain of herpes simplex virus type 1 (HSV) (3) have been described previously. Poliovirus (Mahoney strain) and Sendai virus (strain 52) were obtained from the collection of the Virus Laboratory, Department of Epidemiology, The University of Michigan School of Public Health, Ann Arbor. Poliovirus stocks were prepared by inoculating Vero cells at a multiplicity of infection (MOI) of 1 PFU per cell. At 24 h postinfection, cultures were quickly frozen and thawed twice, the medium was clarified (10,000 × *g*, 10 min), and the supernatant fraction was used as an inoculum without further preparation. Sendai virus was passaged through Vero cells in MEM containing 2 μg of trypsin per ml and without fetal bovine serum (18). Working stocks of Sendai virus were prepared by infecting Vero cells at an MOI of 0.1 PFU per cell. Cells were harvested after 54 h and quickly frozen and thawed twice, and the medium was clarified at 800 × *g* for 10 min. Simian virus 40 (SV-40) was obtained from F. Rapp. SV-40 working pools were prepared by infecting Vero cells at an MOI of 0.1. After 4 days, Vero cultures were quickly frozen and thawed twice, and the medium was clarified at 800 × *g* for 10 min. Newcastle disease virus (NDV) (strain 11914) originally obtained from the American Type Culture Collection was a gift of K. W. Cochran. The virus was prepared as described for Sendai virus except that fetal bovine serum (10%) was present and trypsin was omitted. Vesicular stomatitus virus (VSV) (Indiana strain), a gift of T. J. Schnitzer, was plaque purified five times through Vero cells. A dilute

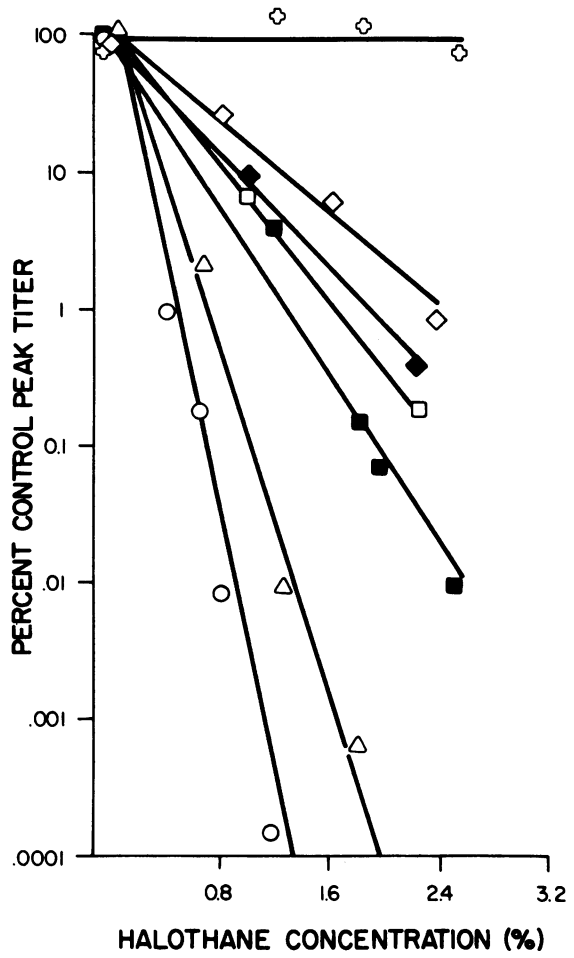

FIG. 1. Concentration response for halothane inhibition of peak virus titers for poliovirus (✣), VSV (◇), SV-40 (◆), Sendai virus (□), HSV(■), NDV (△), and MV (○). In the experiments shown above, the MOIs used were as follows: poliovirus, 1; VSV, 0.1; SV-40, 0.1; Sendai virus, 0.1; HSV, 0.1; NDV, 0.1; MV, 0.1. Identical degrees of susceptibility to halothane exposure were seen when the poliovirus, Sendai virus, or MV MOI was raised 10-fold. The resultant peak (100% control) virus titers (PFU/ml) for the experiments above were: poliovirus, 3.9×10^8; VSV, 1.3×10^9; SV-40, 4.2×10^8; Sendai virus, 1.7×10^8; HSV, 3.6×10^8; NDV, 2.8×10^6; MV, 1.8×10^6.

passage (MOI = 0.1) stock was harvested after 24 h and prepared as described above for NDV.

Exposure of cells to halothane. Vero cell cultures were exposed to halothane vapors, using a Dräger vaporizer as described previously (11), with halothane concentrations being assessed by gas chromatography (11). Halothane was also removed from exposed cultures by changing the culture medium and passing a 95% air-5% CO_2 airstream over the cultures as previously reported (11).

Plaque assay. All viruses were adsorbed for 1 h at 22°C except for SV-40, which was adsorbed for 4 h at 37°C. Infected cultures were washed once with Tris-buffered saline (pH 7.4) after the adsorption period to remove nonadsorbed virus. Culture medium was then added, and the indicated concentration of halothane vapors was added immediately as described previously (11). When halothane-nonexposed, virus-infected Vero cell cultures demonstrated virus-specific cytopathology in 100% of the cells, they were harvested by quick freezing at −70°C along with cultures exposed to the concentrations of halothane indicated in the text. Halothane-exposed cultures were frozen in the presence of the anesthetic. After two freeze-thaw cycles, the number of virus particles in the cell lysate was determined by the plaque assay technique as follows. Infectious MV, NDV, HSV, VSV, and poliovirus titers were measured by the plaque assay technique, using a methylcellulose overlay as previously described (11). Plaques were counted after 1 day for poliovirus, 2 days for VSV, 3 days for HSV, or 7 days for MV and NDV. Plaque assays employing an agar overlay (3) were used for Sendai virus and SV-40. The agar overlay for Sendai virus included 10 μg of trypsin per ml, with serum being omitted (18). A second overlay was added to the SV-40-infected cells after 7 days to sustain cell viability.

RESULTS

RNA viruses. Five RNA viruses were tested for their ability to replicate in Vero cells in the presence of halothane. Figure 1 demonstrates that MV replication was inhibited in a concentration-dependent manner when Vero cells were exposed to the anesthetic. The susceptibility of MV replication to halothane in Vero cells was similar to that previously described for BSC-1 cells (11). The susceptibility of MV replication to halothane in CV-1 cells is considerably greater, however, with total inhibition occurring at 1.2% (unpublished data). The halothane-induced inhibition of NDV replication resembled that of measles virus. Total inhibition of both of these syncytium-producing viruses occurred well within the clinical range of halothane (i.e., ≤2.5%). The replication of a third member of the Paramyxoviridae family, a syncytium-nonforming strain of Sendai virus, was also affected by exposure to halothane, although the inhibition was not complete at the highest concentration tested. The peak titer observed after exposure to 2.2% halothane was reduced by 2.5 orders of magnitude as compared with nonexposed controls. Similarly, the replication of VSV was only moderately inhibited when infected Vero cells were exposed to halothane. A decrease of 2 orders of magnitude in infectious virus titers was observed at 2.5% halothane. Poliovirus replication was not inhibited by halothane at any concentration tested (Fig. 1).

The reversibility of halothane-induced inhibition of replication of the viruses described above is shown in Fig. 2. All RNA viruses whose replication was inhibited by exposure to halothane were able to replicate after the anesthetic was removed. NDV, like MV, began to recover shortly after the removal of the anesthetic, but infectious NDV titers did not approach peak control values until 40 h after removal of the anesthetic. MV and Sendai virus each took 12 to 16 h to recover, whereas VSV titers approached levels of the nonexposed control within 6 to 8 h after halothane removal.

DNA viruses. The replications of HSV and SV-40 were both inhibited by halothane in a concentration-dependent manner. A 2.3% concentration of halothane caused a decrease of 2 to 3 orders of magnitude as compared with control virus titers of SV-40 and a drop in peak virus titers of 4 orders of magnitude with HSV (Fig. 1). After removal of halothane, the infectivity of HSV returned to control levels after 24 h (Fig. 2). However, SV-40 titers were generally not more than 10% of control values after 72 h and never reached more than 20% of the values for nonexposed cultures even 96 h after halothane removal (data not shown).

Effect of pH and nutrients on virus inhibition and recovery. The presence of high $NaHCO_3$ concentrations attenuated the

antiviral effect of halothane slightly. Figure 3 shows that, in the presence of 0.15% $NaHCO_3$ (as compared with a usual concentration of 0.09%), a higher dose of halothane was necessary to totally inhibit MV replication. The halothane-induced inhibition of VSV replication was similarly less dramatic in the more highly buffered medium.

With the viruses whose replication was susceptible to halothane, a complete reversal of halothane-induced inhibition of replication occurred only when culture medium was changed after anesthetic removal. Failure to change the culture medium after anesthetic removal resulted in slightly lower peak titer values for all viruses tested (Table 1). All volatile inhalational anesthetics, including halothane, inhibit respiration (15) with a concomitant increase in lactate production due to enhanced glycolysis (3a, 7). To test whether the resulting lactate excess excreted into the culture medium inhibited recovery, we performed the following experiment. MV-infected cultures grown in the presence of 2.2% halothane were compared with anesthetic-nonexposed control cultures. When MV-infected control cultures of Vero cells demonstrated cytopathology in 100% of the cells, the culture medium was changed in one-half of the halothane-exposed cultures. In the other half of the halothane-exposed cultures, enough $NaHCO_3$ was added to raise the medium pH from 6.7 to 7.5, the pH observed in our anesthetic-nonexposed cultures. When the $NaHCO_3$ concentration was raised to 0.15%, replication of MV resumed at rates which were the same as though the medium had been changed. Furthermore, if the culture medium was changed but HCl was added

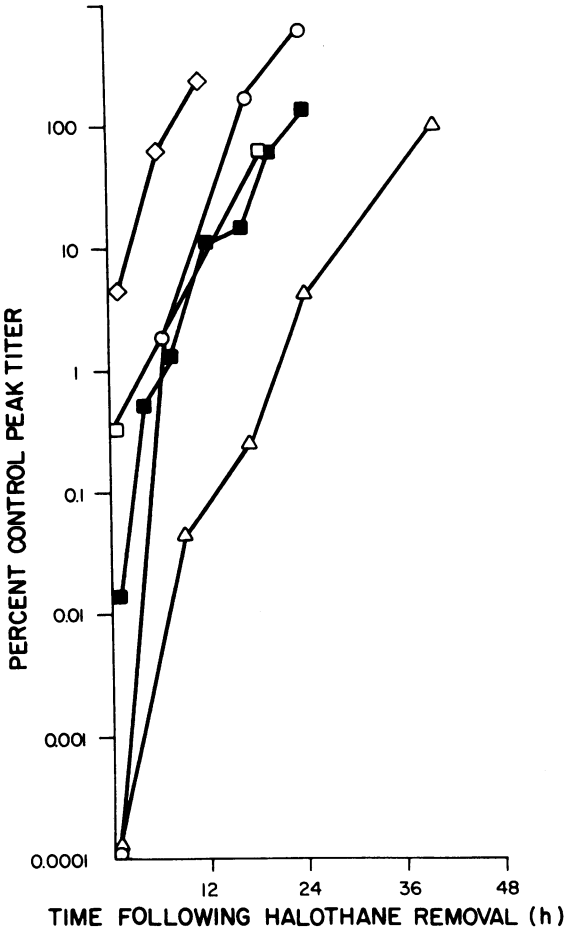

FIG. 2. Recovery of virus replication after halothane removal and culture medium change for VSV (◇), HSV (■), Sendai virus (□), NDV (△), and MV (○). After exposure of virus-infected Vero cells to 2.4% halothane for the period of time necessary for nonexposed, virus-infected cells to demonstrate virus-specific cytopathology in 100% of the cells, the culture medium was replaced with fresh MEM. A 95% air-5% CO_2 airstream was then directed over the cultures for 25 min to eliminate any residual halothane vapors. Infected cultures were then incubated at 37°C, and samples were harvested as described in the text. Shown above are virus titers at the indicated times after halothane removal. Peak (control) virus titers and multiplicities of infection are the same as those shown in Fig. 1.

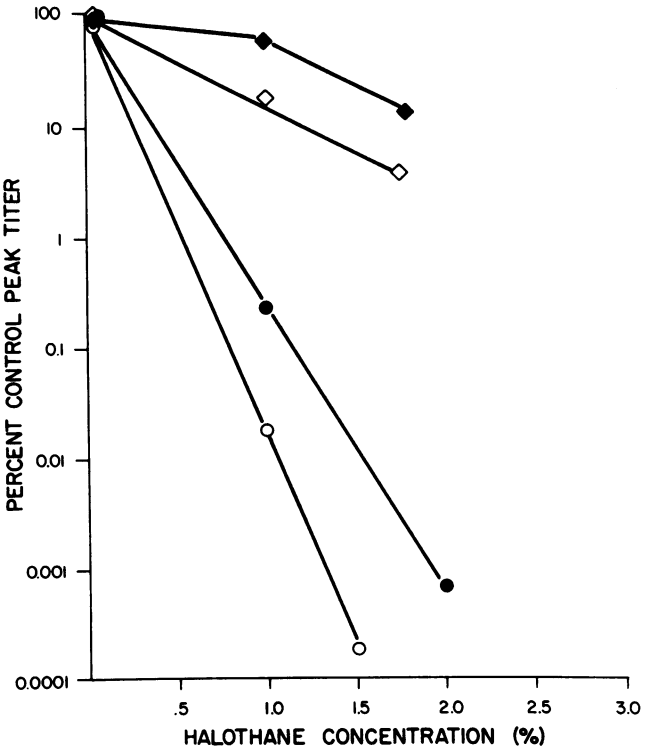

FIG. 3. Concentration response for halothane inhibition on peak titers of VSV in 0.15% (◆) or 0.09% (◇) $NaHCO_3$ or MV in 0.15% (●) or 0.09% (○) $NaHCO_3$. MOIs for this experiment were as described in the legend to Fig. 1, with the peak (100% control) titers for each of the viruses as follows: VSV, 3.3×10^9 and 8.3×10^8 in 0.15 and 0.09% bicarbonate, respectively; MV, 7.9×10^6 and 1.8×10^6 in 0.15 and 0.09% bicarbonate, respectively.

TABLE 1. Effect of medium change on virus recovery

Virus	Nonexposed peak control titer	Virus titer at 2.3% halothane[a]	Peak recovery titer[b]	
			No medium change	Medium changed
MV	2.2×10^6	1.0×10^1	2.1×10^5	4.6×10^6
VSV	1.3×10^9	5.9×10^7	1.1×10^8	3.5×10^9
HSV	8.0×10^8	4.7×10^5	2.8×10^7	3.6×10^9

[a] Titers of halothane-exposed cultures were determined when nonexposed cultures reached their peak titer value.

[b] Recovery titers were determined at 16.5 h after halothane removal for MV, after 12 h for VSV, and after 24 h for HSV.

to lower the pH, recovery was retarded. Recovery was complete at pH 7.1 to 7.5, but at pH 6.8, the recovery was only 9%, whereas at pH 6.3, virus titers were less than 0.002% of those at pH 7.1. Similar results were observed in experiments performed with VSV. Thus, it appears to be the low pH resulting from lactate excretion which inhibits virus recovery and not nutrient depletion or the build-up of other toxic products.

Correlation between susceptibility to halothane and the length of the virus replication cycle. Poliovirus and VSV were not only the least affected but are also the most rapidly growing of the viruses tested. Since halothane is known to inhibit ATP production in exposed cells (15), we exposed Vero cultures to 2.2% halothane 24 h before (and continued during) infection with poliovirus, VSV, and MV to see whether the sensitivity of these three viruses would be altered in cells which had a reduced capacity to generate ATP. Twenty-four hours of 2.2% halothane pretreatment caused no additional inhibition in the replication of any of the viruses. Therefore, the fact that poliovirus and VSV are the least sensitive to halothane exposure is not because they grow too quickly to allow for chronic effects within the cells to accumulate.

DISCUSSION

Halothane is used clinically as a general inhalational anesthetic. One property of all general inhalational anesthetics is that their clinical potency is a linear function of their lipid solubility (8). Therefore, it is interesting that poliovirus, the only RNA virus tested which neither buds from its host cell outer membrane nor contains a membrane envelope, is also the only virus tested which was not susceptible to the antiviral effect of halothane. The replication of two syncytium-forming viruses, MV (a morbillivirus) and NDV (a paramyxovirus), was totally inhibited by clinical concentrations of halothane. A syncytium-nonforming strain of another paramyxovirus, Sendai virus, was not completely inhibited, however. Rather, the replication of Sendai virus, like that of VSV, was partially, albeit significantly, decreased by 2.4% halothane. Since the RNA-containing syncytium-forming viruses as a group were the most susceptible to halothane exposure, anesthetic interaction with the plasma membrane structure and functions required for fusion and giant cell formation must be considered as a site of antiviral activity.

The replication of two DNA viruses, HSV and SV-40, was moderately affected by halothane exposure. Titers of nonenveloped SV-40 were lowered by 2 orders of magnitude, compared with the inhibition of 4 orders of magnitude seen at 2.4% halothane for HSV, which is enveloped. The site of assembly of these DNA viruses is in the nucleus and, at least for HSV, has been shown to be the inner nuclear membrane (16, 17). Preliminary experiments have indicated that the packaging of HSV DNA into nucleocapsid structures, a process which occurs on the nuclear membrane (17), is less efficient in the presence of 2.4% halothane (manuscript in preparation). SV-40 replication may be less sensitive to the antiviral activity of halothane than HSV replication because it does not require the deposition of a membrane envelope for its infectivity, whereas SV-40 replication may be more sensitive to halothane exposure than poliovirus replication because vital assembly sites within the nucleus are more sensitive to halothane than are those cytoplasmic sites required by poliovirus (4).

Poliovirus and VSV were the two most rapidly growing viruses tested in these studies and also the two most resistant to the antiviral action of halothane, whereas HSV is intermediate in its growth rate and sensitivity. The three members of the paramyxovirus family, MV, NDV, and Sendai virus, are relatively slow growing, and two of those, MV and NDV, are the most susceptible to halothane. Although the third member of the paramyxovirus group, Sendai virus, and the slow-growing SV-40 both exhibit only intermediate susceptibility to halothane exposure, there also appears to be a general correlation between the time necessary for the virus to complete a replicative cycle and the capacity of halothane to disrupt that replication.

Halothane-induced inhibition in virus replication was readily reversible after removal of the anesthetic. All RNA viruses (Fig. 2, open symbols) and HSV (closed squares) recovered totally after halothane removal, and the rate of recovery essentially reflected their growth rate normally seen in Vero cells. The one exception was MV, which reached peak titers within 15 h after removal of the anesthetic; this rate faster than that predicted for a virus which takes 36 h to produce peak titers and cytopathology in 100% of the cells under the standard culture conditions of our laboratory. The short recovery time observed after removal of the anesthetic-induced block may reflect only the time necessary for MV to assemble and cause cell fusion rather than the additional time necessary for virus protein and RNA synthesis. The fact that SV-40 replication did not totally recover after 96 h may be a result of its long replication cycle in Vero cultures. Alternatively, a nuclear alteration vital to SV-40 synthesis may be susceptible to halothane exposure, possibly sufficient to disrupt the SV-40 lytic cycle.

Pressures of 100 to 200 atm (10,110 to 20,220 kPa) are capable of reversing clinical anesthesia and the membrane alterations caused by lipophilic anesthetic agents presumably by either compressing the membrane (13) or by extruding the anesthetic molecule from the membrane (8, 9). It appears that certain virus products such as the nucleocapsid protein are synthesized in halothane-exposed Vero cells (manuscript in preparation), but that these products are not assembled into functional virus particles (11). The recent observation (1) that, in the presence of 100 atm of helium, MV can assemble in what is otherwise a concentration of halothane which completely inhibits virus replication suggests that the plasma membrane or possibly other compressible structures within the cell may play a role in the MV assembly process. Similarly, it demonstrates that a compressible site such as the plasma membrane of the cell may be a vital target for the antiviral activity of halothane.

Halothane appears to affect more than one site within the cell. It also disrupts mitochondrial respiration in a concentration-dependent and reversible manner (15), causing exposed cells to utilize glycolytic metabolism more extensively and resulting in the production of increased amounts of lactate (3a, 7). A slight enhancement of ca. 1 order of magnitude in the antiviral effect of halothane was observed in MV- or VSV-infected cultures which were grown in MEM containing 0.09% as compared with 0.15% $NaHCO_3$. Although it is possible that the slight increase in susceptibility to low pH observed reflects the ability of the viruses to maintain their infectivity in slightly acidic conditions, it is not likely that the antiviral nature of halothane is due to the low pH of the medium caused by its presence. Four different viruses (poliovirus, VSV, HSV, and MV) maintained their susceptibilities to halothane exposure when culture medium was buffered so that no significant pH change occurred, although the concentration of halothane necessary to produce an equivalent amount of inhibition increased. For example, total inhibition of measles virus replication at pH

7.4 still occurred but at 2.3% rather than at 1.8% halothane, as was seen at pH 6.7.

Low pH appeared to retard virus recovery. Virus replication resumed after anesthetic removal and after either changing of the culture medium or adjusting of the pH of unchanged medium. If the pH of unchanged medium was not raised or the pH of fresh medium was lowered by the addition of HCl after halothane inhibition, the degree of MV recovery was diminished accordingly. No recovery occurred at pH 6.3, recovery was partial at pH 6.7 to 6.8 (the lowest pH observed in the media of Vero cultures treated with 2.2% halothane [3a]), whereas recovery was complete at pH 7.1 or greater. These results argue against disruption of mitochondrial respiration being a primary cause of the antiviral effect of halothane. We have recently demonstrated that although 100 atm of pressure is capable of reversing clinical anesthesia (8) and the halothane-induced inhibition of MV replication of Vero cells (1), it does not reverse the halothane-induced inhibition of respiration in rat liver mitochondria (5) or lactate production in Vero cells (3a). This also suggests that the inhibition of mitochondrial respiration is not the mechanism by which general anesthetics work.

The in vitro studies described here correlate to some degree with previous results seen in vivo. Diethyl ether anesthesia increases survival in canines infected with distemper virus (6), a synctium-forming morbillivirus related to MV. Similarly, mice inoculated with several encephalitis-causing togaviruses (enveloped, positive stranded, and RNA containing) also demonstrate decreased mortality when anesthetized with diethyl ether during the course of the infection (19), and mice infected with influenza virus (enveloped, segmental, and negative-stranded RNA) demonstrate an enhancement in survival when exposed to enflurane and, to a more limited degree, halothane (10). However, neither of the diseases produced by poliovirus or rabies virus (a rhabdovirus similar to VSV) was ameliorated by exposure of infected mice to diethyl ether (19). Therefore, exposure to general inhalational anesthetics generally appears to decrease mortality in animals infected with those classes of virus which showed the greatest degree of susceptibility to halothane in this study. However, it does not necessarily follow that anesthesia therapy may be a beneficial means of treating acute virus infections. Previous studies (12) have shown that, after the removal of halothane, MV-infected cells in cultures produce less than full-length nucleocapsids, which resemble defective forms that are implicated as possible etiological agents of latent virus diseases, including those of the central nervous system (14). Although it has never been firmly established that these altered nucleocapsid forms produce a clinical disease, further studies should be conducted before we can decide conclusively the effects of anesthesia on patients harboring virus infections.

Anesthesiologists by training do not electively give anesthesia to patients harboring upper respiratory tract infections. However, preliminary results in humans have revealed no increased mortality or morbidity in patients harboring such infections after surgery and halothane-nitrous oxide anesthesia (manuscript in preparation). Results described here, along with previous animal studies (see above), indicate that the type of virus, animal host, or anesthetic administered (10) may all affect the course of virus replication during clinical anesthesia. Studies aimed at elucidating the molecular basis of virus inhibition by general anesthetics are currently being pursued in this laboratory. It is hoped that these studies will point to particular sites within the cell in which the virus and anesthetic interact, enabling us to understand how virus replication and assembly may be affected during clinical anesthesia. Furthermore, we hope to identify the particular site(s) of the cell which is affected by anesthetic exposure, providing further insight into the mechanism of action of general inhalational anesthetics.

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LITERATURE CITED

1. **Bedows, E., and P. R. Knight.** 1983. Pressure reversal of halothane's antiviral effect. Anesthesiology **59**:109–112.
2. **Bedows, E., and F. E. Payne.** 1981. Host cell factors involved in the production of slowly sedimenting nucleocapsids in measles virus infected cells. J. Virol. **37**:103–108.
3. **Bedows, E., K. M. K. Rao, and M. J. Welsh.** 1983. Fate of microfilaments in Vero cells infected with measles virus and herpes simplex type 1 virus. Mol. Cell. Biol. **3**:712–719.

3a. **Brabec, M. J., E. Bedows, B. A. Davidson, and P. R. Knight.** 1984. Effect of general anesthetics and pressure on aerobic metabolism in monkey kidney cells. Anesthesiology **61**:43–47.

4. **Caliguiri, L. A., and I. Tamm.** 1970. Characterization of poliovirus-specific structures associated with cytoplasmic membranes. Virology **42**:112–122.
5. **Cohen, P. J.** 1983. Effect of hydrostatic pressure on halothane-induced depression of mitochondrial respiration. Life Sci. **32**:1647–1650.
6. **Donovan, C. A.** 1968. The influence of diethyl ether inhalation on experimental canine distemper. Vet. Med. Small Anim. Clin. **63**:345–347.
7. **Fink, B. R., and G. E. Kenny.** 1968. Metabolic effects of volatile anesthetics in cell culture. Anesthesiology **29**:505–516.
8. **Franks, N. P., and W. R. Lieb.** 1982. Molecular mechanisms of general anesthesia. Nature (London) **300**:487–493.
9. **Galla, H. J., and J. R. Trudell.** 1980. Asymmetric antagonistic effects of an inhalation anesthetic and high pressure on the phase transition temperature of dipalmitoyl phosphatidic acid bilayers. Biochim Biophys. Acta **599**:336–340.
10. **Knight, P. R., E. Bedows, M. L. Nahrwold, H. F. Maassab, C. Smitka, and M. T. Busch.** 1983. Alterations in influenza virus pulmonary pathology induced by diethyl ether, halothane, enflurane, and pentobarbital anesthesia in mice. Anesthesiology **58**:209–215.
11. **Knight, P. R., M. L. Nahrwold, and E. Bedows.** 1980. Anesthetic action and virus replication: inhibition of measles virus replication in cells exposed to halothane. Antimicrob. Agents Chemother. **17**:890–896.
12. **Knight, P. R., M. L. Nahrwold, and E. Bedows.** 1981. Inhibiting effect of enflurane and isoflurane anesthesia on measles virus replication: comparison with halothane. Antimicrob. Agents Chemother. **20**:298–306.
13. **Lever, M. J., K. W. Miller, D. W. M. Paton, and E. B. Smith.** 1971. Pressure reversal of anaesthesia. Nature (London) **231**:368–371.
14. **Morgan, E. M., and F. Rapp.** 1977. Measles virus and its associated diseases. Bacteriol. Rev. **41**:636–666.
15. **Nahrwold, M. L., and P. J. Cohen.** 1975. Anesthetics and mitochondrial respiration, p. 25–44. *In* P. J. Cohen (ed.), Molecular aspects of anesthesia. E. A. Davis Co., Philadelphia.
16. **Sambrook, J.** 1978. The molecular biology of papova viruses, p. 589–672. *In* D. P. Nayak (ed.), The molecular biology of animal viruses, vol. 2. Marcel Dekker, Inc., New York.

17. **Schwartz, J., and B. Roizman.** 1969. Concerning the egress of herpes simplex virus from infected cells. Electron microscope observation. Virology **38**:42–49.
18. **Shibuta, H., M. Akami, and M. Matsumoto.** 1971. Plaque formation by sendai virus of parainfluenza group, type I on monkey, calf kidney and chick embryo cell monolayers. Jpn. J. Microbiol. **15**:175–183.
19. **Sulkin, S. E., C. Zarafonetis, and A. Goth.** 1946. Influences 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.