Comparative Effects of Anesthetics on the Viability and Integrity of *Escherichia coli* ML30

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Cells of *Escherichia coli* ML30 in a mineral salts medium were exposed to dichlorodifluoromethane (f-12), cyclopropane, halothane, or Ethrane at concentrations of 1.25, 0.2, 0.04, and 0.008× saturation for times up to 1,200 min, and at temperatures in the range of 2 to 37°C. When any of these anesthetics were applied for 300 min at 1.25× saturation, a substantial decrease in number of survivors occurred. Halothane was most bactericidal, cyclopropane and Ethrane were moderately bactericidal, and f-12 was least bactericidal. At saturation values of less than 1.0, none of the four anesthetics had an appreciable effect on viability of *E. coli*. Greatest increases in cell permeability occurred when anesthetics were used at saturation values of 1.25, and permeability changes generally decreased as the concentrations of the chemicals were reduced. In many instances, anesthetics in the vapor state caused significant increases in cell permeability but little or no loss of viability. This indicated that a close relationship did not exist between loss of viability and increased permeability. All four anesthetics caused *E. coli* to lose substantial and similar amounts of compounds absorbing at 260 nm. Release of compounds absorbing at 260 nm generally increased as the saturation value of a given chemical was increased. Halothane, Ethrane, and cyclopropane but not f-12 caused lysis of *E. coli* ML30. Considering all results, *E. coli* ML30 was damaged more by halothane or cyclopropane than by f-12 or Ethrane. When f-12 was applied at a saturation value of 1.25, the bactericidal effect on *E. coli* was much greater at 37 or 22°C than at 12 or 2°C.

Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), Ethrane (1,1,2-trifluoro-2-chloroethyldifluoromethyl ether), cyclopropane, and fluoro-carbon-12 (dichlorodifluoromethane [f-12]) have in common an ability to induce narcosis in animals. The first three chemicals are used as clinical anesthetics for humans whereas f-12 is not used clinically because of its undesirable side effects. f-12 is used commercially as a refrigerant, an aerosol propellant, and a freezing medium for unpackaged foods.

Although anesthetics have been used extensively, their effects on bacteria, other than on population size, have received scant attention. When used in the vapor state at low concentrations, halothane can inhibit growth of numerous bacteria including *Salmonella typhimurium*, *Bacillus licheniformis*, *Staphylococcus albus*, *Escherichia coli*, *Micrococcus lysodeikticus*, *Corynebacterium* sp., *Brucella melitensis*, and a hemolytic streptococci (3, 7, 11, 17). Halothane is usually bactericidal to microorganisms when used in a liquid state or in a vapor state at high concentrations (1, 17; S. L. Hegeman, Ph.D. thesis, Univ. of California, San Francisco, 1969).

f-12 inactivates a wide range of bacteria, yeasts, and molds, especially when used in the liquid state (13, 15). Fluorocarbons similar to f-12 were recently studied by Van Auken et al. (16) to determine their potential as food preservatives. They stored milk, eggs, fish, and shrimp in the presence of trichlorofluoromethane, dichlorofluoromethane, or monochlorodifluoromethane for periods up to 12 weeks at room temperature. f-12 was the most effective chemical tested; it retarded substantially the growth of microorganisms in milk when used at a concentration of about nine times that needed for saturation, and it had similar effects on eggs, shrimp, and fish when used at a concentration of about 0.1 to 0.35 times the amount needed for saturation.

Little published information is available concerning the effects of Ethrane and cyclopropane on microorganisms.
Thus, the effects of individual anesthetic gases on microorganisms have been studied to only a limited degree and the comparative effects of different anesthetics on various properties of microorganisms are even less well known.

The purpose of this study was to compare the effects of the anesthetics halothane, Ethrane, cyclopropane, and f-12 on the viability and integrity of E. coli during various conditions of time, anesthetic concentration, and temperature. The results are important from two standpoints: (i) the microbiological effects of clinical anesthetics are of obvious concern to the medical profession because of the obvious merit of using anesthetics that dissuade growth of microorganisms, and because the effects of anesthetics on microorganisms may provide a measure of the relative toxicities of anesthetics to humans; and (ii) since anesthetic gases are relatively nontoxic to humans, are easily removable (sparingly soluble in water, moderately volatile) from most kinds of samples (metals, biological specimens), and therefore are easily reusable, they may be useful as bactericidal agents in food processing and treatment of equipment.

**MATERIALS AND METHODS**

**Organisms and media.** E. coli ML30 (supplied by J. Garver of the University of Wisconsin) was grown at 37°C in the glucose-mineral salts-trace salts medium of Kruwich et al. (9) and incubated to the late logarithmic phase (5 h at 37°C) of growth. Cells were harvested by low speed centrifugation (about 2,000 × g), washed once with cold sterile distilled water, and finally resuspended in the desired concentration in mineral salts medium. To enumerate viable cells, samples were suitably diluted in 0.1% peptone water, plated in duplicate in plate count agar (Difco), and incubated at 37°C for 48 h, and the resulting colonies were visually counted.

**Chemicals.** Test chemicals included f-12 (food grade, du Pont), cyclopropane (trimethylene, Liquid Carbonic Co., U.S. Pharmacopeia grade), halothane (CF₂CHClBr, Fluothane, Ayerst Laboratories, anesthetic grade; distilled before use to remove thymol), and Ethrane (F₂HCOF₂CHClIF, Ohio Medical Products, research grade, 99.9% pure).

**Dispensing chemicals.** Liquid Ethrane and halothane were dispensed with either a 2-ml or 50-μl syringe, depending on the amount desired. When relatively large amounts of these liquids were needed they were dispensed in an undiluted form. Small amounts were handled by saturating water with the chemical and dispensing appropriate amounts of the aqueous solution. The precision of adding these chemicals was ±6% by weight (95% confidence limit).

Cyclopropane and f-12 were metered in liquid form from a high-pressure buret into capped aerosol cans (13). The precision of adding these chemicals was ±8% by weight (95% confidence limit).

Chemicals were superimposed on the normal atmosphere existing in the headspace of the can.

**Means of expressing chemical concentrations.** If a toxic substance is present at equilibrium concentrations in all phases of a system, then the chemical potential of this substance will be equal in all phases. According to Ferguson (5), the chemical potential of any toxic substance that functions by a physical mechanism is closely related to its toxic potency. Since values for chemical potential are not easily obtained, Ferguson suggested that a useful approximation in the case of solutions is the term $C/C_0$, where $C$ is the weight of toxic substance dissolved in the liquid phase and $C_0$ is its solubility in the liquid phase at the same temperature and pressure. Since some of the chemical concentrations used in this study exceeded saturation, Ferguson’s definition was modified slightly to accommodate this condition. Thus, $C$ is defined as the weight of chemical present in the liquid phase and $C_0$ is the solubility of the chemical in the liquid phase at the same temperature and pressure. Chemical concentrations ($C/C_0$) of 1.25, 0.2, 0.04, and 0.008 × saturation were chosen for this study.

Based on literature values, use of the ideal gas equation, and some experimental values, the weights of chemical necessary to saturate a sample containing 10 ml of water and 87 ml of vapor space at 37°C were: 3.68 g for f-12, 1.46 g for cyclopropane, 0.442 g for halothane, and 0.332 g for Ethrane. Amounts of f-12 necessary for saturation at various temperatures were: 1.51 g at 2°C, 2.01 g at 12°C, 2.83 g at 22°C, and 3.68 g at 37°C.

**General procedures.** Aerosol cans (5.1 by 5.4 cm, 97-ml capacity) with double epoxy linings and stainless-steel caps (without dip tubes) served as sample containers. Cans and caps were sterilized separately in an autoclave. A 10-ml quantity of the culture was added aseptically to each aerosol can and the can was immediately loosely covered with a sterile aerosol cap. When f-12 or cyclopropane was being tested, the cap was first crimped in place and the chemical was then added by means of a high-pressure buret (13). Halothane and Ethrane were added by carefully lifting the cap and injecting the chemical into the can with a syringe. The cap was immediately crimped in place to prevent loss of the chemical.

Samples were prepared in triplicate and incubated at 37 ± 2°C for the concentration studies, and at 2, 12, 22 or 37 ± 2°C for the temperature studies. During treatment the samples were agitated at 120 horizontal oscillations per min on a platform shaker (model 6000, Eberbach Corp., Ann Arbor, Mich.).

**Other procedures.** Preparation of an E. coli lysate, measurement of percent lysis, measurement of release of compounds absorbing at 260 nm (ultraviolet [UV] compounds), and measurement of release of [*¹⁴C]α-methylglucoside (MG) were done as described previously (14).

**RESULTS**

**Influence of various concentrations of anesthetics on E. coli ML30 at 37°C.** Anesthetics...
were tested at concentrations of 1.25, 0.2, 0.04, and 0.008× saturation. Data in Fig. 1 show the effects of various kinds and concentrations of anesthetics on the viability of *E. coli*. When anesthetics were applied for 300 min at a concentration of 1.25× saturation (liquid present), substantial decreases in the number of survivors always occurred. At this concentration, halothane inactivated *E. coli* most rapidly and most effectively (fewest survivors). Cyclopropane and Ethrane inactivated *E. coli* somewhat less rapidly than halothane but more rapidly than f-12. Cyclopropane, Ethrane, and f-12 were about equal in effectiveness over a period of 300 min; all treatments resulted in about 1,000 survivors/ml. At concentrations below saturation the anesthetics had almost no observable effect on viability of *E. coli* with the possible exception of halothane at 0.2× saturation for 300 min. Results of this study clearly indicate that halothane was the most effective against *E. coli* of the four chemicals tested and that none of the chemicals was effective unless used at a concentration above 0.2× saturation.

Somewhat similar results were reported by Barry et al. (1). They grew *E. coli* cells on blood agar and then treated the cultures with halothane. Total inactivation of *E. coli* was achieved after a 10-min exposure to liquid halothane (conditions of agitation and temperature were not given), whereas vapor-state halothane had no bactericidal effect. Other workers have found

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**Fig. 1.** Viability of *E. coli* ML30 at 37 C as influenced by various kinds and concentrations of anesthetics. Variability symbols represent the ranges of three sample replicates. For data without variability symbols the variability was too small to indicate. Data for cyclopropane at 1.25× and 30 min were regarded as unreliable and were therefore deleted.
that halothane in the vapor state is mainly bacteriostatic rather than bactericidal. Wardley-Smith and Nunn (17) reported that halothane at a vapor concentration of 7 to 8% reduced the growth rate of E. coli by 50%, whereas 60% (vol/vol) vapors of halothane completely inhibited growth of E. coli at 37 C.

Horton et al. (7) reported results somewhat contrary to those above. They studied cultures of E. coli situated on membranes of cellulose acetate and found that halothane present as 3.1% of the vapors reduced survivors by 55% after a 2-h treatment. Complete sterilization was achieved in 2 h with a vapor concentration of 16% halothane. It should be noted, however, that the Horton study was purposely designed to approximate conditions that would exist on the surfaces of anesthetic equipment. Thus, samples were probably exposed to low relative humidities and this could have increased the sensitivity of E. coli to halothane.

The effect of anesthetics on permeability of E. coli is shown in Fig. 2. Greatest increases in permeability occurred when the chemicals were used at a concentration of 1.25 x saturation. At this concentration, halothane and cyclopropane caused a more rapid and more complete release of MG than did the other two anesthetics. Increases in permeability also occurred when E. coli was treated with anesthetics at concentra-

![Graphs showing the effect of anesthetics on the release of [14C]α-methylglucoside from E. coli ML30 during incubation at 37 C. Variability symbols represent ranges of three observations.](image)
Fig. 3. Effect of anesthetics on release of 260-nm absorbing compounds from E. coli ML30 during incubation at 37 C. Variability symbols represent the range of three sample replicates. For data without variability symbols, the variability was too small to indicate. D, Deleted because the data were excessively variable.

Fig. 4. Effect of anesthetics on lysis of E. coli ML30 during incubation at 37 C. Variability symbols represent ranges of three sample replicates. For data without variability symbols, the variability was too small to indicate.

samples treated with f-12). After 30 min, halothane, cyclopropane, and Ethrane at concentrations of 1.25x saturation were significantly more damaging than these same chemicals at lesser concentrations. However, the differences were less pronounced after 300 and 1,200 min than at 30 min. Halothane, even at the lowest concentration, was especially effective in lysing E. coli.
Considering all results in Fig. 1 to 4, it is apparent that \textit{E. coli} was damaged more by halothane and cyclopropane than by either f-12 or Ethrane. Halothane was somewhat more damaging than cyclopropane, whereas the effects of Ethrane and f-12 were nearly equal.

Some additional tests were done to determine the effect of xenon on \textit{E. coli}. Cultures of \textit{E. coli} at 22°C were exposed to xenon at 25 and 100 lbs/in² (saturation value unknown) for 0, 30, 300, and 1,200 min. Treated cultures exhibited no detectable loss of viability or change in integrity as compared to untreated cultures (data not shown). Had changes occurred, it could have been concluded that xenon, as well as the anesthetics previously tested, functioned primarily by physical mechanisms. Since no changes were observed, it must be concluded that the anesthetics studied here functioned either by chemical means or by physical mechanisms not possible with xenon at the pressures used.

Hegeman (Ph.D thesis, Univ. of California, San Francisco, 1969) obtained similar results with xenon. She found that \textit{E. coli}, subjected to xenon at a pressure of 65 lbs/in², survived to the same extent as untreated samples. However, aerobic and anaerobic growth of \textit{E. coli} at 37°C were inhibited reversibly by pure vapors of xenon.

**Effect of f-12 on \textit{E. coli} at various temperatures.** The purpose of this study was to determine the extent to which responses of \textit{E. coli} ML30 to f-12 were affected by temperature. Concentrations of 1.25 and 0.04× saturation were chosen because they provide, respectively, the liquid and vapor states of the chemical and were known from previous studies to provide meaningful results. Temperatures of 2, 12, 22, and 37°C were selected since they adequately cover the range extending from just above freezing to the optimum growth temperature for \textit{E. coli}. Viability and integrity of cells of \textit{E. coli} were measured by the same four tests previously used.

Effects of temperature on \textit{E. coli} exposed to f-12 at 1.25× saturation are shown in Fig. 5. During a 30-min exposure, temperature exerted a small and usually insignificant effect. During treatment for 1,200 min, exposure to 22 and 37°C resulted in substantially fewer survivors than exposure to 12 or 2°C. Other properties of \textit{E. coli} were affected only moderately by temperature during a treatment for 1,200 min, and

![Graphs showing viability, release of \(\alpha\)-methyl glucoside, release of UV materials, and lysis for \textit{E. coli} ML30 treated and untreated with f-12 at various temperatures.](image)

**Fig. 5.** Effect of temperature on responses of \textit{E. coli} ML30 to f-12 at a concentration of 1.25× saturation. Variability symbols represent the ranges of three sample replicates. For data without variability symbols, the variability was too small to indicate.
the pattern observed was not entirely predictable. It is noteworthy that three tests at 1,200 min (release of UV compounds, release of MG, and lysis) clearly indicate greater damage to *E. coli* at 2 than at 12 C. The same behavior was exhibited by the 1,200-min control sample in the MG study. This phenomenon was investigated further and is discussed later.

When the concentration of f-12 was reduced to 0.04× saturation, it was clear (results not shown) that the range of temperatures used had essentially no effect on damage to *E. coli* by f-12. In the MG study at 0.04× saturation it was again noteworthy that permeability increases in cells treated for 30 min were slightly greater at 2 than 12 C.

The effects of halothane, Ethrane, and cyclopropane on *E. coli* varied with temperature in a manner similar to that observed for f-12 (data not shown).

Additional studies were done with halothane at a concentration of 0.2× saturation, and it was found that leakage of MG from *E. coli* was significantly greater at 2 than 12 C for both control and treated samples (data not shown). This confirms the results obtained with f-12. Initial incubation of *E. coli* at 15 rather than 37 C did not change the result just mentioned. Thus chilling damage alone apparently was not responsible for the results obtained since *E. coli* grown at 15 C would be more likely to resist chilling damage than would cells grown at 37 C (4).

It is possible that cold osmotic shock could be responsible for the results obtained. Patching and Rose (12) subjected *E. coli* to cold osmotic shock and found that they could not incorporate MG, indicating damage to the transport system. Cells grown at 15 and 37 C were damaged equally suggesting that temperature of growth did not influence cell resistance to cold osmotic shock. This is in accord with results obtained in this study.

Flock et al. (6) conducted a study somewhat similar to that reported here. They treated *Photobacterium phosphoreum* with vapors of halothane and observed decreasing light output at increasing temperatures. However, they concluded that the result was not caused by increased potency of halothane as the temperature was raised, but rather by the increased solubility of halothane at the higher temperatures. This result conflicts with data in Fig. 5 wherein increasing temperature increased the potency of f-12 even though the saturation value of f-12 was held constant. It should be noted, however, that experimental details of the two studies differed in several respects and this could account for the discrepancy.

**DISCUSSION**

In this study, four anesthetics were tested at approximately equal saturation values (C/C*) and their effects on *E. coli* were determined by several means. When the chemicals were used at a saturation value of 1.25 (1.25× saturation) and viability was used as an index of toxicity, halothane was far more bactericidal than the other three anesthetics. At lower concentrations, all four anesthetics exhibited approximately the same bactericidal effect, but this is probably true only because small bactericidal effects were observed. Thus, at a concentration of 1.25× saturation, these results appear to violate the rule suggested by Ferguson (5) and Brink and Posternak (2) that equal values of C/C* result in equal effectiveness. Furthermore, it is unlikely that this violation of the proposed rule can be accounted for on the basis of experimental errors associated with solubility data and addition procedures.

Mullins (10) stated that the rule of equal potency at equal values of P/P* (where P is the partial pressure of the chemical in the vapor space and P* is the saturation vapor pressure of the pure chemical at the same temperature; a concept equivalent to C/C*) would be valid only for chemicals of approximately the same molecular volume and that corrections are necessary when comparing compounds of different molecular volumes. He concluded that the smaller the molecular volume, the smaller the P/P* value needed to induce anesthesia. The molecular volumes of the four anesthetics used are listed in Table 1. Based on this information, cyclopropane should have the greatest biological activity at a given saturation value. It is clear that the effectiveness of halothane cannot be accounted for on the basis of its molecular volume. Differences in atomic compositions of anesthetics used in this study may have had a bearing on the results obtained. The anesthetic potency of a halogenated hydrocarbon generally increases with the number and molecular volume

| Anesthetic | Molecular Vol (ml/mol) | Temp (C) | Source |
|------------|-----------------------|----------|--------|
| Cyclopropane | 62                  | 20       | Mullins (10) |
| f-12       | 76                   | 30       | Formula* |
| Halothane  | 106                  | 30       | Formula* |
| Ethrane    | 121                  | 25       | Formula* |

*Calculated from the formula: molecular volume = molecular weight/density.
weight of the component halogen atoms. Thus, anesthetic potencies of halogenes are as follows: I > Br > Cl > F (8). Of the three halogenated hydrocarbons used in this study, iodine was present in none and bromine was present only in halothane, and this may account for its potency. Differences in the number of carbon atoms or the number of halogen atoms per molecule did not appear to greatly influence toxicities of the compounds tested.

It is unlikely that impurities in halothane were sufficient to account for its unusual effectiveness. Anesthetic grade halothane contains thymol as a preservative but this was removed by distillation before use. Furthermore, no impurities were detected by gas chromatography in the halothane used.

The finding that halothane exhibits unusual effectiveness is in general accord with results of White and Dundas (18). They found that the light output of P. phosphoreum was inhibited more effectively by halothane than by cyclopropane.

Results reported here, and earlier results by Van Auken et al. (16), suggest that treatment of food with anesthetics may hold some promise as a means of preservation. For example, food such as meat, fruits, and vegetables conceivably could be briefly dipped in liquid halothane to inactivate vegetative microorganisms present on the surface. If surface microorganisms behave like E. coli did in this study, it should be possible to markedly reduce the population of microorganisms on surfaces of foods, and thereby extend the keeping qualities of these foods. Since anesthetics in the vapor state were relatively ineffective against E. coli, it is likely that liquid anesthetics would be needed for this type of application.

Because anesthetics are reasonably nontoxic, quite inert, moderately volatile, and sparingly soluble in water, they appear unusually well suited for use with food. Volatility and limited solubility would facilitate separation and recovery of the chemicals after use and this is important from a cost standpoint. The volatility, inertness, and low toxicity of these chemicals also are advantageous from the standpoint of food safety. It should be relatively easy under realistic commercial conditions to remove the chemicals sufficiently so that any residues would pose no hazard to the health of the consumer.

Since the results observed in this study cannot be accurately extrapolated to situations involving food preservation, additional research is necessary to determine the feasibility of preserving foods by means of anesthetics.

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