The Effects of Anesthetic Agents on Murine Pulmonary Bactericidal Activity

Elliot Goldstein, M.D., Edwin S. Munson, M.D.,† Carroll Eagle, B.A.,‡ Richard W. Martucci, B.A.,‡ Paul D. Hoeprich, M.D.§*

The effects of anesthesia on pulmonary antibacterial defense mechanisms of the murine lung were assessed quantitatively. Following infection with aerosols of ^{32}P-labelled *S. aureus*, mice were anesthetized to approximately equivalent levels with halothane, pentobarbital, methoxyflurane or cyclopropane. After four hours of anesthesia, the animals were sacrificed and the rate of radiophosphorus removal and intrinsic pulmonary bactericidal activity were determined by established techniques. Anesthesia with methoxyflurane or cyclopropane significantly depressed pulmonary bactericidal activity. Anesthesia with halothane or pentobarbital did not affect bactericidal activity. None of the anesthetics altered the rate of physical removal of bacteria. The adverse effects of methoxyflurane and cyclopropane could not be attributed to changes in body temperature or blood-gas composition. (Key words: Alveolar macrophage; Pulmonary infections; MAC; Halothane; Methoxyflurane; Cyclopropane; Pentobarbital.)

PNEUMONIA continues to be a common complication after major surgery.[1-3] The mechanisms responsible are poorly understood but have been attributed to aspiration of gastric contents,[3-5] atelectasis,[3, 6, 7] tracheal trauma,[1-2] defective cough mechanism,[2] and contaminated aerosol equipment.[8, 9] Recent experimental studies have demonstrated the importance of intrinsic pulmonary antibacterial defenses in keeping the lungs bacteria-free.[10, 11] Bacteria which normally are inhaled and reach the lower respiratory tract are rapidly phagocytized by the pulmonary macrophage system.[11] Experimentally induced metabolic abnormalities such as acidosis,[12] hypoxia,[13] and renal failure[14] affect this intrinsic defense system adversely, presumably by interfering with pulmonary macrophage function. Since anesthetic agents may produce metabolic dysfunction[15-18] and in certain instances inhibit the phagocytic activity of the peritoneal macrophage,[19, 20] they may also alter the antibacterial defense systems of the murine lung. This study demonstrates significant decreases in pulmonary bactericidal activity following the prolonged administration of anesthetic concentrations of cyclopropane and methoxyflurane, but not halothane or pentobarbital.

Methods

Eight-week-old male white Swiss mice weighing 25 to 30 g were used in all experiments. The animals were housed in plastic cages and fed Purina Lab Chow and water *ad lib.*

PREPARATION OF BACTERIAL AEROSOL

Staphylococcus aureus, strain 566, was cultured and labelled with phosphorus-32 (^{32}P) by the method of Green and Kass.[11] In this procedure the test organism is inoculated into 25 ml of a phosphorus-free culture media containing 1.0 mc of ^{32}P. After 16 hours of growth at 37 C in a shaker water bath, the labelled cells are removed by centrifugation, washed, and resuspended in 8.0 ml of phosphate buffer, *p*H 7.4. Five ml of this suspension were used for aerosolization.

* Assistant Professor of Medicine.
† Associate Professor of Anesthesiology.
‡ Research Bacteriologist.
§ Professor of Medicine.

Received from the Section of Infectious and Immunologic Diseases, Department of Internal Medicine, and the Department of Anesthesiology, School of Medicine, University of California, Davis, California 95616. Accepted for publication December 15, 1970. Supported by Public Health Service Grant APO 1239 from the National Air Pollution Control Administration, Health Sciences Advancement Award RR06138-02, and a grant from the California Tuberculosis Association. Portions of these data were presented at the Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, October 1970.

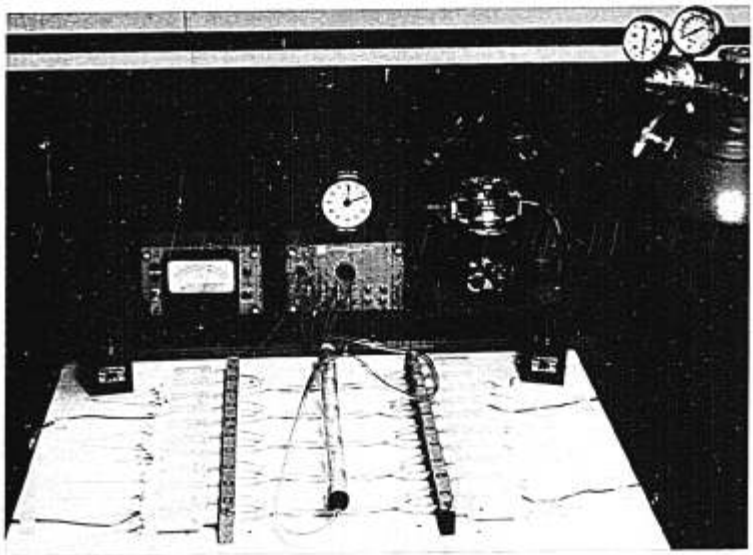

FIG. 1. Apparatus used to anesthetize mice with halothane or methoxyflurane.

ANIMAL EXPOSURE

Groups of 30 or 40 mice were infected with aerosols of radiolabelled (^{32}P) *S. aureus* in an aerosol apparatus designed to produce uniform inhalation dosages of infecting bacteria.[10] Ten mice were sacrificed immediately after exposure and the radioisotope concentrations in their lungs measured. The remainder were divided into groups of ten and either anesthetized for four hours with one of the agents being studied or returned to their cages as controls. A heat lamp was used to maintain the body temperatures of the anesthetized mice during the experimental period. The exact temperatures were monitored in approximately half of the animals with rectal temperature probes. A standardized tail-clamping technique was used to assess depth of anesthesia.[21] At the end of the four-hour experimental period, arteriolized tail-vein blood was obtained in a heparinized capillary tube and measurements of *p*H, P_{CO_2} and P_{O_2} were made with appropriate electrodes. The animals were then sacrificed with ether, the lungs were removed aseptically and homogenized, and samples were measured for ^{32}P activity and bacterial concentrations.[22] Sacrificing the animals with ether does not affect the numbers of viable bacteria present within the lungs (unpublished data). Radioisotope concentrations were determined by liquid scintillation counting.[23] Pour-plate methods were used for bacterial enumeration. Radioisotope counts were corrected for background and dilution and expressed as number of counts in the total lung tissue of each animal. The bacterial counts were corrected for dilution and expressed as the number of bacteria in the total lung tissue of each animal. Since the loss of radiolabel from the lungs during the four-hour experimental period is small, the ^{32}P count in mice killed four hours after exposure to the aerosol is an index of the number of staphylococci inhaled initially. In order to establish the number of bacteria represented by ^{32}P radioactiv-

TABLE 1. The Effects of Halothane Anesthesia on the Antibacterial Defense Mechanisms of the Murine Lung

	Experimental Group	^{32}P Count in Lungs (Mean ± SE)	Bacterial Count in Lungs (Mean ± SE)	Bactericidal Activity (Per Cent) (Mean ± SE)*
Set 1	Control 0 hr (10)†	16,217 ± 1,379		
	Control 4 hr (10)	13,118 ± 1,083	5,175 ± 965	92.0 ± 1.1
	Halothane 4 hr (9)	12,085 ± 740	3,388 ± 445	94.0 ± 0.8‡
Set 2	Control 0 hr (10)	17,234 ± 1,096		
	Control 4 hr (10)	12,079 ± 1,197	11,375 ± 3,819	88.1 ± 4.5
	Halothane 4 hr (10)	16,492 ± 2,082	12,579 ± 2,846	90.4 ± 1.6‡

Overall Radiophosphorus Removal and Bactericidal Activity

Experimental Group	^{32}P Removal (Per cent) (Mean ± SE)	Bactericidal Activity (Per Cent) (Mean ± SE)
Control 4 hr (20)	25.7 ± 5.9‡	89.9 ± 2.3‡
Halothane 4 hr (19)	27.8 ± 2.6	92.2 ± 1.0

* K values for the two sets were: set 1 = 4.8; set 2 = 8.3.
† Number in brackets is the number of animals studied.
‡ Not significant, $P > 0.05$.

ity, an Andersen sampler was attached to the aerosol chamber during the period of infection. Quantitative measurements of radioactivity and bacteria were made from the 1.0–2.0-micron sampling plate. A ratio of bacterial concentration to radioactivity was computed and designated the aerosol labelling ratio: K. The mathematical expression of pulmonary bactericidal activity for an individual mouse could then be computed from the formula:

$$\text{Per cent bactericidal activity} = \left[1 - \frac{\text{bact ct}_{1\text{ hr}}}{^{32}\text{P}_{1\text{ hr}} \times \text{K}}\right] \times 100$$

Per cent bactericidal activity is the rate at which bacteria are killed within the lungs, where bact $ct_{4\text{ hr}}$ equals bacterial count at four and $^{32}P_{4\text{ hr}}$ equals radiophosphorus count at four hours. The analysis of variance method was used to test for significance of differences. It should be noted that the data obtained from the animals sacrificed immediately after infection (0 hr) do not enter into these calculations. These data were used to compute the rate of physical removal of inhaled bacteria from the lung by means of the formula:

$$\text{Per cent bacterial removal} = \left[\frac{^{32}\text{P}_{0\text{ hr}} - ^{32}\text{P}_{4\text{ hr}}}{^{32}\text{P}_{0\text{ hr}}}\right] \times 100$$

where $^{32}P_{0\text{ hr}}$ equals the radiophosphorus counts of the lungs of mice sacrificed immediately after aerosolization and $^{32}P_{4\text{ hr}}$ equals radiophosphorus counts of lungs from animals sacrificed four hours after aerosolization. This formula assumes a firm binding of the radioisotope to the bacteria, and that changes in radioisotope concentration are due to physical removal of bacteria with the attached label from the lung. Previous studies have confirmed a firm binding of radiotracer to bacteria and shown that separation of radiolabel from the attached bacteria is small.[14] Therefore, the rate of removal of the radiophosphorus label is an index of the rate of physical removal of inhaled bacteria. These data were analyzed by the theorem of Wilks.[24]

Approximately equivalent levels of anesthesia were established with all agents by adapting a technique used for the determination of MAC, the minimum alveolar concentration of anesthetic.[21, 25, 26]

In the present study, halothane and methoxyflurane in air were delivered at flow rates

>4 l/min into 15 to 20 transparent chambers which enclosed the heads and chests of the mice (fig. 1). Anesthesia was induced within ten minutes using either 3 per cent halothane or 2 per cent methoxyflurane. The concentration of anesthetic gas was then reduced in a stepwise manner while testing the responses to tail-clamp stimulation with a small surgical hemostat. Within ten to 15 minutes the anesthetic levels were reduced such that 30 to 50 per cent of the mice responded with movement on tail clamping; thus, equivalent depths of anesthesia were assumed.

Cyclopropane was administered in an oxygen–nitrogen mixture from calibrated flowmeters. Gases were delivered at a flow rate of 4l/min into a 14-liter container into which the mice were introduced. Soda lime granules were used to prevent an increase in the carbon dioxide concentration within the chamber. Cyclopropane at a concentration of 30 per cent was administered for ten minutes. MAC has been shown to be 22.3 per cent (unpublished data of ESM). Since tail clamping was not feasible in this system, cyclopropane concentration was decreased to levels between 20 and 25 per cent following induction of anesthesia. Oxygen concentration in the outflowing gas was monitored with a Beckman (Model D) Oxygen Analyzer and was maintained at 21 per cent.

Pentobarbital anesthesia was induced with an initial intraperitoneal dose of 50 mg/kg body weight. Subsequent doses of 30 mg/kg were administered whenever the animals showed spontaneous movement. The average cumulative pentobarbital dosage over the period of the experiment was 100 mg/kg/hour.

Results

The data for the two experiments in which mice were anesthetized with halothane are presented in table 1. The rates of physical removal of radiophosphorus and pulmonary bactericidal function were equivalent for the an-

TABLE 2. The Effects of Methoxyflurane Anesthesia on the Antibacterial Defense Mechanisms of the Murine Lung

	Experimental Group	^{32}P Count in Lungs (Mean ± SE)	Bacterial Count in Lungs (Mean ± SE)	Bactericidal Activity (Per Cent) (Mean ± SE)*
Set 1	Control 0 hr (10)†	20,930 ± 2,520		
	Control 4 hr (9)	17,423 ± 917	20,923 ± 6,696	86.5 ± 4.4
	Methoxyflurane 4 hr (9)	19,796 ± 2,058	54,211 ± 8,092	69.2 ± 4.0‡
Set 2	Control 0 hr (10)	15,634 ± 2,279		
	Control 4 hr (9)	13,307 ± 1,922	5,754 ± 1,061	91.5 ± 1.0
	Methoxyflurane 4 hr (7)	10,720 ± 1,694	8,551 ± 1,582	82.1 ± 3.6‡
Set 3	Control 0 hr (10)	14,455 ± 1,691		
	Control 4 hr (9)	11,501 ± 1,285	8,166 ± 2,660	87.7 ± 3.7
	Methoxyflurane 4 hr (6)	11,375 ± 1,728	8,225 ± 2,254	89.6 ± 1.6
Set 4	Control 0 hr (10)	17,346 ± 2,286		
	Control 4 hr (10)	11,190 ± 984	13,125 ± 2,820	83.0 ± 3.1
	Methoxyflurane 4 hr (8)	13,860 ± 1,929	21,805 ± 2,940	75.6 ± 2.0‡

Overall Radiophosphorus Removal and Bactericidal Activity

Experimental Group	^{32}P Removal (Per Cent) (Mean ± SE)	Bactericidal Activity (Per Cent) (Mean ± SE)
Control 4 hr (37)	22.0 ± 6.6	87.1 ± 1.7 (37)
Methoxyflurane 4 hr (30)	15.5 ± 8.6	78.0 ± 2.1 (30)‡

* K values for the four sets were: set 1 = 9.1; set 2 = 5.0; set 3 = 6.6; set 4 = 6.5.
† Number in brackets is the number of animals in each group.
‡ Significant, $P < 0.05$.

TABLE 3. The Effects of Cyclopropane Anesthesia on the Antibacterial Defense Mechanisms of the Murine Lung

	Experimental Group	^{32}P Count in Lungs (Mean ± SE)	Bacterial Count in Lungs (Mean ± SE)	Bactericidal Activity (Per Cent) (Mean ± SE)*
Set 1	Control 0 hr (10)†	9,772 ± 588		
	Control 4 hr (10)	7,070 ± 748	15,890 ± 4,578	90.4 ± 1.9
	Cyclopropane 4 hr (8)	7,391 ± 686	43,225 ± 9,699	71.2 ± 7.3‡
Set 2	Control 0 hr (10)	5,929 ± 462		
	Control 4 hr (10)	5,432 ± 420	14,385 ± 5,744	89.9 ± 3.2
	Cyclopropane 4 hr (7)	5,226 ± 161	35,199 ± 4,967	71.1 ± 4.2‡
Set 3	Control 0 hr (10)	12,070 ± 760		
	Control 4 hr (10)	11,060 ± 1,145	19,810 ± 3,851	93.0 ± 1.3
	Cyclopropane 4 hr (7)	10,601 ± 1,060	49,501 ± 7,221	81.7 ± 2.6‡

Overall Radiosphosphorus Removal and Bactericidal Activity

	^{32}P Removal (Mean ± SE)	Bactericidal Activity (Per Cent) (Mean ± SE)
Control 4 hr (30)	15.1 ± 8.7	91.1 ± 1.3
Cyclopropane 4 hr (22)	16.4 ± 8.5	74.5 ± 3.1‡

* K values for the three sets were: set 1 = 10.5; set 2 = 23.3; set 3 = 26.0.
† Number in brackets is the number of animals in each group.
‡ $P < 0.01$.

esthetized and control animals. Each group removed approximately 25 per cent of the inhaled radioisotope and killed 90 per cent of the inhaled bacteria during the four-hour experimental period.

Methoxyflurane data are shown in table 2. In three of the four aerosol experiments, pulmonary bactericidal activity was significantly impaired in the methoxyflurane-treated animals ($P < 0.05$). The overall mean bactericidal activity was 78.0 per cent for the anesthetized mice, compared with 87.1 per cent for controls ($P < 0.01$). The rates of pulmonary radioisotope removal were similar, averaging 15.5 per cent for methoxyflurane-treated mice and 22.0 per cent for controls.

TABLE 4. The Effect of 10 Per Cent Cyclopropane on Murine Pulmonary Bactericidal Activity

	Experimental Group	^{32}P Count in Lungs (Mean ± SE)	Bacterial Count in Lungs (Mean ± SE)	Bactericidal Activity (Per Cent) (Mean ± SE)*
Set 1	Control 4 hr (10)†	10,658 ± 560	3,535 ± 784	95.0 ± 1.0
	Cyclopropane 4 hr (10)	9,625 ± 1,040	13,685 ± 2,905	77.1 ± 4.6‡
Set 2	Control 4 hr (8)	8,250 ± 1,379	5,425 ± 959	85.4 ± 3.2
	Cyclopropane 4 hr (9)	9,383 ± 847	13,338 ± 1,806	71.8 ± 2.3‡

Mean Bactericidal Activity

Control (18)		90.7 ± 1.9
Cyclopropane (19)		74.6 ± 2.7‡

* K values for the two sets were: set 1 = 3.2; set 2 = 5.2.
† Number in brackets is the number of animals studied.
‡ $P < 0.01$.

TABLE 5. The Effects of Pentobarbital and Methoxyflurane Anesthesia on the Antibacterial Defense Mechanisms of the Murine Lung

	Experimental Group	^{32}P Count in Lungs (Mean ± SE)	Bacterial Count in Lungs (Mean ± SE)	Bactericidal Activity (Per Cent) (Mean ± SE)*†
Set 1	Control 0 hr (10)‡	9,895 ± 396		
	Control 4 hr (10)	9,982 ± 753	12,215 ± 2,646	92.2 ± 1.8
	Pentobarbital 4 hr (10)	9,496 ± 938	12,705 ± 2,937	91.2 ± 2.1
	Methoxyflurane 4 hr (10)	10,458 ± 1,403	41,895 ± 16,649	77.8 ± 5.9
Set 2	Control 0 hr (10)	2,790 ± 191		
	Control 4 hr (10)	3,136 ± 408	65,275 ± 11,865	90.8 ± 1.2
	Pentobarbital 4 hr (10)	2,636 ± 264	74,540 ± 35,028	89.1 ± 4.1
	Methoxyflurane 4 hr (10)	2,657 ± 257	86,310 ± 17,353	86.2 ± 1.7

Overall Radiophosphorus Removal and Bactericidal Activity

Experimental Group	^{32}P Removal (Mean ± SE)	Bactericidal Activity (Per Cent) (Mean ± SE)†
Control 4 hr (20)	−3.4 ± 19.6	91.5 ± 1.0
Pentobarbital 4 hr (20)	4.7 ± 19.3	90.2 ± 2.3
Methoxyflurane 4 hr (20)	−3.3 ± 22.4	82.0 ± 3.1

* K values for the two sets were: set 1 = 15.5; set 2 = 115.2.
† $P > 0.05$ for comparisons of controls and pentobarbital;
$P < 0.05$ for comparisons of controls and methoxyflurane.
‡ Number in brackets is the number of animals in each group.

During the four-hour period of exposure to cyclopropane, 20 to 30 per cent of the mice died, probably as a result of failure to maintain adequate airways within the anesthetizing container. As shown in table 3, pulmonary bactericidal activity was markedly decreased in the anesthetized mice of each experimental set ($P < 0.01$): the overall bactericidal activity was 74.5 per cent for cyclopropane-treated mice and 91.1 per cent for controls ($P < 0.01$). The rates of removal of radiophosphorus were similar in the two groups.

Since the responses to cyclopropane anesthesia varied among individuals in these experiments and some mice might have been hypoxic during the four-hour period of anesthesia, the experiment was repeated using 10 per cent cyclopropane (table 4). Anesthesia did not result; indeed, the mice were hyperexcitable during most of the experimental period. Pulmonary bactericidal activity was depressed to the same extent as noted in the animals anesthetized with 20 per cent cyclopropane ($P < 0.01$).

The data for the two experiments in which pentobarbital anesthesia was used are shown in table 5. Methoxyflurane was used as a positive control in these experiments. Anesthesia with pentobarbital did not affect pulmonary bactericidal activity. However, methoxyflurane did affect pulmonary bactericidal activity, and the animals anesthetized with this agent had diminished bactericidal activity rates when compared with either of the other two groups ($P < 0.05$). The rates of pulmonary radiophosphorus removal were similar for the three groups.

Temperatures and *p*H, P_{CO_2} and P_{O_2} values for all of the experimental groups were similar. These data were combined and are presented in table 6. The temperatures of mice anesthetized with halothane averaged 36.8 C; methoxyflurane, 35.9 C; pentobarbital, 35.9 C. These temperatures are within the normal range of 35.2 to 37.8 C.[27] Anesthesia with cyclopropane resulted in an abnormally high rectal temperature of 38.9 C. The constant activity of the controls and the mice exposed to 10 per cent cyclopropane prevented continual monitoring of temperatures.

TABLE 6. Rectal Temperatures and Blood *p*H, P_{CO_2} and P_{O_2} Values of Mice Exposed to Various Anesthetic Agents

Experimental Group	Temperature (C) (Mean ± SD)	*p*H (Mean ± SD)	P_{CO_2} (mm Hg) (Mean ± SD)	P_{O_2} (mm Hg) (Mean ± SD)
Control	*	7.28 ± .08	41.4 ± 8.2	64.3 ± 14.4
Halothane	36.8 ± 0.96	7.30 ± .05	33.7 ± 10.1	
Methoxyflurane	35.9 ± 1.24	7.23 ± .06	44.6 ± 6.6	61.3 ± 9.3
Cyclopropane	38.9 ± 0.31	7.30 ± .09	38.8 ± 6.6	66.5 ± 17.7
Cyclopropane, 10 Per Cent		7.22 ± .13	44.4 ± 12.0	65.3 ± 13.5
Pentobarbital	35.9 ± 1.04	7.24 ± .04	48.4 ± 4.5	72.2 ± 7.6

* Normal temperature 35.2–37.8 C.[37]

Blood *p*H and P_{CO_2} values were consistent with a mild degree of metabolic acidosis in all experimental groups. Degrees of blood oxygenation, as determined by the P_{O_2} values in arteriolized tail-vein blood, were also similar in all groups.

Discussion

These experiments demonstrate that anesthesia with cyclopropane or methoxyflurane significantly diminished the ability of the murine lung to kill inhaled bacteria. Neither halothane nor pentobarbital affected this intrinsic pulmonary defense mechanism. Although there were variations in radiophosphorus removal rates among mice anesthetized with the various agents, these differences were not significant. Most of the radiophosphorus is bound to the inhaled staphylococci and, therefore, these results indicate that in this experimental model mucociliary function is not impaired by exposure to anesthetics for four hours.

Several metabolic factors (depth of anesthesia, *p*H, P_{CO_2}, P_{O_2} and body temperature) were measured, since they might bear on the observed impairment in pulmonary bactericidal function following cyclopropane or methoxyflurane anesthesia. None of these factors appeared to be responsible for the inhibition of antibacterial function. The anesthetic levels in the cyclopropane- and methoxyflurane-treated mice were similar to the levels maintained in the halothane- and pentobarbital-treated animals. Furthermore, cyclopropane in subanesthetic dosages inhibited pulmonary bactericidal activity. Disturbances in acid–base balance also were not significant, since the mild to moderate degree of metabolic acidosis present in the treated mice was equivalently present in corresponding control animals. In addition, previous experiments have shown that much more severe degrees of metabolic acidosis are necessary before bactericidal function is impaired.[12]

The role of hypoxia in the present experiments is difficult to assess. Hypoxia has been shown to diminish murine pulmonary bactericidal activity.[13] Undoubtedly, some of the anesthetized mice experienced periods of diminished oxygenation. However, for the following reasons it is unlikely that the observed abnormalities in pulmonary bactericidal function were caused by hypoxia. First, the measurements of arteriolized tail-vein blood did not show differences in oxygen tension when anesthetized mice with impaired bactericidal function were compared with untreated controls. Second, unanesthetized mice exposed to 10 per cent cyclopropane and 21 per cent oxygen were not hypoxic, and these animals had severe impairment of pulmonary bactericidal function. Third, mice anesthetized with pentobarbital had blood oxygen tensions quite similar to those of mice anesthetized with methoxyflurane and cyclopropane but did not have defective bactericidal function.

Hypothermia, which also has been shown to diminish pulmonary bactericidal function,[13] was not a factor in these experiments. The mice were kept euthermic by means of a heat lamp throughout the four-hour experimental period. The significance of the mild hyperthermia which occurred in the mice anesthetized with cyclopropane is not known.

It is possible that the adverse effects of methoxyflurane and cyclopropane resulted from direct cellular toxicity. Previous studies have demonstrated that intrapulmonary bactericidal

activity is due to phagocytosis by pulmonary macrophages and that this system is the primary host defense against inhaled bacteria.[11] Since the anatomic position of the pulmonary macrophage ensures intimate exposure to inhaled anesthetic, this key element in defense might suffer if toxicity occurs. At present only a few investigations of anesthetic effects upon macrophage function have been reported, and to our knowledge neither cyclopropane nor methoxyflurane has been studied.

Barbiturates do not appear to affect pulmonary defense systems. Laurenzi and Guarneri investigated the *in vivo* effects of barbiturates on pulmonary defense mechanisms and found a negligible reduction in bacterial clearance.[2a] Our experimental findings confirm their observations. Halothane has been reported to enhance murine susceptibility to intraperitoneal infection with *Salmonella typhimurium*.[19] This anesthetic-induced alteration in host defense was attributed to defective leukocytic function.[20] The difference between these results and ours may be due to differences in the microorganisms studied or the methods used to evaluate host defense.

Ethanol seems to diminish pulmonary antibacterial activity through inhibition of phagocytic function.[29] Ether also may increase susceptibility to bacterial infecton by depressing cellular phagocytosis.[20, 21] Therefore, although the available evidence is meager, it appears that anesthetic agents are capable of adversely affecting cellular defense systems and in this manner enhancing susceptibility to pulmonary bacterial infection.

The authors thank Dr. Harry Brandt of the Department of Mechanical Engineering and Dr. Charles Franti of the Department of Epidemiology and Preventive Medicine for their aid in these studies. The halothane (Fluothane) and methoxyflurane (Penthrane) used in these experiments were graciously donated by the Ayerst and Abbott Companies, respectively.

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