

The Anesthesia Machine and Circle System Are Not Likely to be Sources of Bacterial Contamination

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Patients who had upper respiratory tract gram-negative bacillary colonization and noncolonized patients were followed through surgical procedures to determine what bacterial organisms would be deposited in anesthesia apparatus. Anesthesia machines were cultured for bacteria in many locations before and after each surgical procedure. Six machines in routine operating room use were studied after use on six colonized patients and nine uncolonized patients. Sixteen corrugated tubes from unopened packages served as controls. The results indicated that the machines remained free of bacteria of patient origin. Levels of contamination were only slightly higher in the expiratory tubing, and the bacterial species most commonly recovered were environmental in origin. Even after periods of anesthetic administration as long as six hours in patients heavily colonized with gram-negative bacilli, contamination of the anesthesia apparatus with the colonizing organisms did not occur. Intentional contamination of a sterilized anesthesia machine with two gram-negative organisms confirmed the clinical observations. Analysis of oxygen and nitrous oxide gas sources for bacteria had negative results. Basic hygienic management of anesthesia machines will ensure safety from the standpoint of cross-infection. (Key words: Equipment, anesthesia machine; Equipment, circle system; Equipment, bacterial contamination.)

BACTERIAL CONTAMINATION of anesthesia machines as a source of pulmonary infection and cross-infection among anesthetized patients has been the subject of controversy for many years.¹⁻⁶ It has been assumed that anesthesia apparatus may harbor pathogenic microorganisms that are a possible threat to the anesthetized patient. This assumption has fostered additional controversy about decontamination procedures.⁷ Furthermore, items of disposable equipment, including endotracheal tubes, corrugated tubing, masks, bags, and in-line bacterial filters, have all been introduced on the basis of this assumption. We wished to understand whether the anesthesia machine and circle system could be a significant source of bacterial contamination or vector in cross-infection in anesthetized patients, and if so, to determine how best to prevent or interrupt this cycle.

Consequently, we studied possible sources of bacterial contamination in the anesthesia machine and circle system before and after use in both colonized and uncolonized patient groups.

Methods and Materials

Patients were placed into two groups, those colonized with gram-negative bacilli and those not colonized. Six patients comprised the colonized group and nine comprised the uncolonized group. Colonization was defined as isolation of more than ten colonies of gram-negative bacilli from material from the throat or sputum on two or more consecutive days. The tracheas of five of the six colonized patients had been previously intubated, and they were scheduled for tracheostomy. The uncolonized group comprised patients scheduled for elective surgical procedures whose throat and sputum cultures and chest roentgenograms were normal. In the uncolonized group, either the tracheas were intubated or anesthesia was administered by mask. Cultures of material from the noses and throats of all patients were repeated just prior to operation.

All anesthetics were administered with one of six Boyle-type anesthesia machines equipped with a semi-closed circle system and in-line ventilator.‡ The anesthesia machines used in this study were in routine use and received no special treatment other than the usual normal cleaning, which consisted of wiping the surface of the machine with a damp cloth, followed by an alcohol wipe. Soda lime was used in the CO₂ absorber and changed prior to each use of the machine. A constant-volume ventilator§ was used for patients requiring intraoperative ventilation. Disposable corrugated tubing, bag, plastic "Y" piece, and elbow connector¶ were used for all patients. A sterile rubber mask or red rubber endotracheal tube was used for all uncolonized patients.

In the uncolonized group anesthesia was induced with thiopental, 2.5-3.0 mg/kg, followed by succinylcholine 80-100 mg, when the trachea was intubated. Anesthesia was maintained with enflurane except

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‡ Drager Model 50, Harris-Lake, Inc., Cleveland, Ohio.

§ Ohio Model 800, Ohio Medical Products, Madison, Wisconsin.

¶ Dupaco, Inc. San Marcos, California. Manufacturer makes no statement attesting to the sterility of the tubes, which must therefore be considered unsterile.

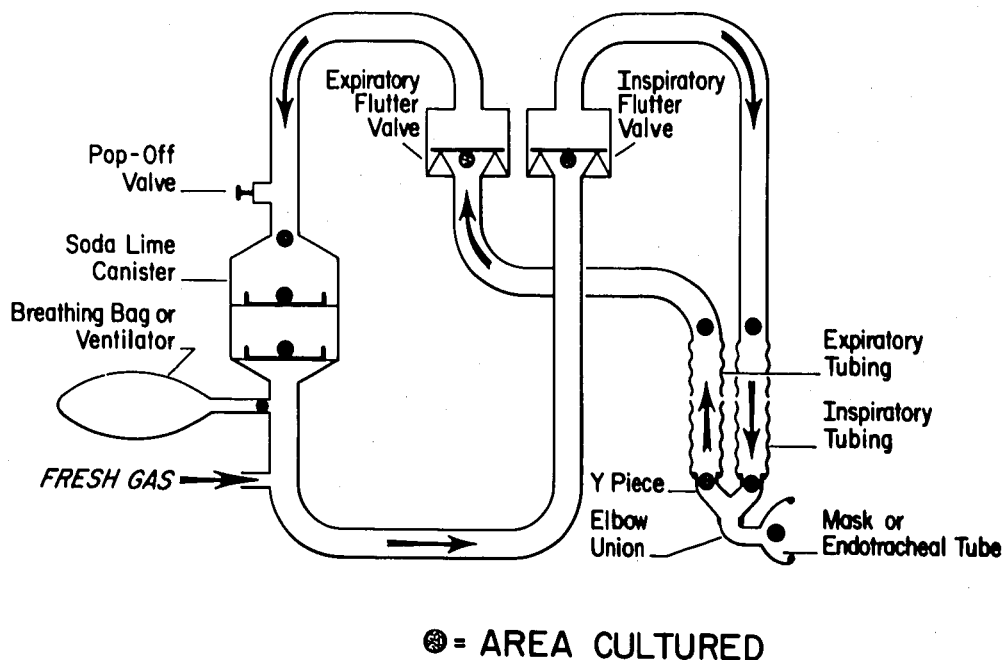


FIG. 1. Anesthesia circle system and the areas sampled for bacteria. Arrows indicate direction of the flow of gases through the circle system. Note that the soda lime canister of the CO₂ absorber was sampled by the placement of petri dishes containing sheep blood agar facing the flow of gases. This is indicated by the two "U"-shaped symbols.

for one patient, who received halothane. All but one patient in the colonized group received enflurane; the one exception received nitrous oxide-morphine anesthesia. All anesthetics were administered using 6 l/min total gas flow, usually consisting of equal flows of nitrous oxide and oxygen. Oral and nasal airways were not used.

Before each use, cultures were taken from six locations on each anesthesia machine: inspiratory port, expiratory port, reservoir bag port, inspiratory valve leaflet (under side), expiratory valve leaflet (under side), and the condensate at the bottom of the CO₂ absorber (fig. 1). Moist areas were sampled with a dry, sterile swab. Dry areas were sampled with a swab moistened in Stuart's transport medium. After sampling, each specimen was placed in holding medium (Stuart's transport medium) and plated immediately or refrigerated. Two blood agar plates were placed within the CO₂ absorber on top of the packed soda lime (one on each pack before each use) so that the agar surface faced towards the direction of the gas flow. The pH of the absorber condensate was measured with indicator paper before and after each surgical procedure.

Hand cultures of anesthesiologists were taken, before they came in contact with the patient, by washing both hands with sterile water and impressing each hand onto a 150-mm Mueller-Hinton plate with 5 per cent sheep blood added. After each machine's initial culturing, disposable anesthesia tubing was connected to the machine; the distal end of the tubing was left covered until ready for

use. Each tube was marked so that it could be identified as either inspiratory or expiratory. The ends that connected to the "Y" piece were also marked. As the patient entered the operating room, settling plates were uncovered and placed at the level of the patient's airway for the duration of the procedure. Immediately following each procedure, tubings, including "Y" piece and elbow joint, were removed from the machine, plugged with sterile gauze, and immediately transported to the laboratory for culturing. The anesthesia machine was again cultured as described above. After extubation of the trachea the endotracheal tube was cultured.

Samples were plated using standard bacteriologic technique. Tubing was treated in the following manner: 14 segments were marked off and numbered consecutively, beginning with the segment closest to the "Y" piece. Each segment measured approximately 6.4 cm and consisted of ten corrugations. The segments were cut from the tubing using aseptic technique. Each segment was split, spread open, and the entire inside surface cultured with a swab moistened in sterile saline solution. The swab was then placed in a tube containing 1.0 ml sterile saline solution. After agitation to remove microorganisms, the swab was compressed against the side of the tube. The saline solution was decanted onto a blood plate containing trypticase soy agar with 5 per cent sheep blood cells and spread out confluent with a sterile glass spreader. Eighteen corrugated tubes taken from unopened packages were also tested in this manner, and comprised the control

group. All bacteriologic plates were incubated overnight at 35 C and at room temperature for three additional days. Colony-forming units were counted at 24, 48, and 72 hours. Gram-negative bacilli were identified by the criteria of Edward and Ewing⁸ for enteric organisms and Weaver, Tatum and Hollis for nonenteric species.⁹ The API enteric identification system** was used throughout for speciation. Other microorganisms were identified by Gram stain and colonial morphology. Staphylococci were subjected to the coagulase test. The presence of fungal species was noted; however, speciation was not attempted.

Colony counts were ranked according to the following scheme: 1 to 9 colony-forming units were designated 1+; 10 to 99 colony-forming units were designated 2+; more than 100 colony-forming units were designated 3+.

To investigate dissemination of a known inoculum of bacteria through a circle system, an anesthetic circle system identical to those used in the operating room was sterilized with ethylene oxide. The expiratory port of this system was inoculated with an overnight culture of *Enterobacter cloacae* (1 ml, 10⁸-10⁹ organisms/ml). The circle system was completed with disposable corrugated tubing, "Y" piece, and elbow connector, which was sealed with a sterile stopper. Sterile water, 15 ml, was added to the bottom of the CO₂ absorber as a source of moisture. A mixture of nitrous oxide and oxygen, 3 l/min of each, was allowed to flow through the system for three hours. Pressure in the circle system was regulated to 15 cm H₂O using the pop-off valve. The circle system was cultured, from ten sampling sites (fig. 1), every 30 minutes during the three-hour period. Following the three-hour period, disposable tubing was divided into segments and cultured as described above. This study was repeated using *Flavobacterium* sp., a gram-negative organism commonly found in hospital water supplies.¹⁰ Methods used for quantitative and qualitative bacteriologic studies were similar to those described above.

To determine how much contamination was contributed to the circle system by gases from central hospital sources, an Andersen Air Sampler¹¹ was interposed between the anesthesia machine outlet and the circle system inlet. The gases were sampled during each of three consecutive 45-minute intervals for a total of 135 minutes using a constant gas flow of 6 l/min. Another sample was collected over a total of 423 minutes using similar gas flows.

Statistical analyses were performed to compare

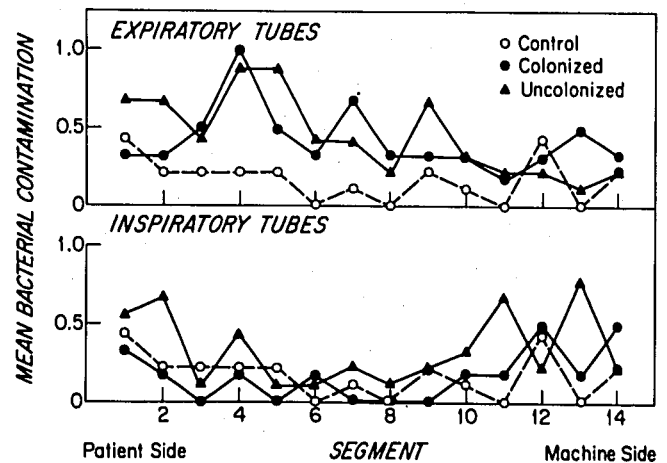


FIG. 2. Results of bacteriologic sampling of individual 6.4-cm segments comprising the expiratory and inspiratory corrugated tubes of the circle system in colonized and uncolonized patients and a control group. Mean bacterial contamination never exceeded 1.0 (1 to 9 colonies/segment). There was no statistically significant difference between mean levels of contamination of controls and the expiratory or inspiratory corrugated tubing. Bacterial contamination consisted primarily of environmental species, including micrococci, staphylococci, and bacilli.

distributions of microbial contamination within the corrugated tubing. Tests for standard deviation, coefficient of variation, and trend analysis were also done. Data were subjected to two-way analysis of variance, followed by multiple-sample comparison. Duncan's test for distribution-dependent data and the Kruskal-Wallis test, a nonparametric analysis, were performed.

This study was carried out with the approval of the Beth Israel Hospital Committee on Clinical Investigations and New Procedures and Forms of Therapy.

Results

The mean level of bacterial contamination for all segments was 1+ or less (1 to 9 colonies) (fig. 2). Segments that were uncontaminated (no growth) were included in the determination of mean bacterial contamination. The three most common contaminants isolated from all groups were (in order of predominance): *Micrococcus* species, *Staphylococcus* species, and *Bacillus* species. Fungal contaminants were also recovered. Gram-negative bacilli, specifically *Acinetobacter calcoaceticus*, *Flavobacterium* species, and *Pseudomonas aeruginosa*, were found in corrugated tubing used on two patients colonized with these organisms. Twenty-six colonies of *Pseudomonas aeruginosa* were found in segment 1 of the expiratory tube used on a patient colonized with this organism

** Analytab Products Inc., Plainview, New York.

after a 75-minute tracheostomy procedure. One colony of *Acinetobacter calcoaceticus* was found in segment 10 and one colony of *Flavobacterium* was recovered in segment 6 of an expiratory tube used on a patient colonized with both organisms after a 120-minute tracheostomy procedure. Gram-negative bacilli were recovered from corrugated tubing used on three uncolonized patients; *Acinetobacter calcoaceticus* was isolated in low numbers from expiratory tubes used on two patients (three and ten colonies), and one colony of *Pseudomonas maltophilia* was recovered from a single segment of expiratory tubing used on the third patient. None of these three patients had these organisms cultured from the upper airways prior to operation. Control tubing from previously unopened packages was found to be contaminated with small numbers of bacteria and fungi. Species of bacteria recovered from control tubing were similar to those identified in corrugated tubing from both colonized and uncolonized patients, i.e., *Bacillus* species, *Staphylococcus* species, and *Micrococcus* species, as well as fungal contaminants.

Statistical analyses of these data indicate that the amount of microbial contamination in each corrugated tubing segment was not affected by its position. Testing for significant trend yielded no consistent linear or curvilinear dependence on segment number. Thus, analysis of the data suggests that contamination patterns are consistent with a totally random distribution of microorganisms along the corrugated tubing.

No positive culture was recovered from any site in the machines used on the six colonized patients. Following administration of anesthesia to the uncolonized group only three positive cultures were obtained from six anesthesia machines: the first from the condensate at the bottom of the CO₂ absorber (*Staphylococcus epidermidis*), the second from the ventilator connection port (*Staphylococcus epidermidis*), and the third from the expiratory port (unidentified gram-negative bacillus).

An average of 0.18 colony-forming units per minute was recovered from settling plates. Species recovered were predominantly environmental types, including *Micrococcus* species, *Staphylococcus* species, and a rare gram-negative bacillus.

Bacterial growth obtained from samples taken at consecutive 30-minute intervals from the expiratory port following its deliberate contamination with *Enterobacter cloacae* showed a progressive decrease in contamination over three hours. None of the indicator organisms was recovered from any other area of the apparatus or from the corrugated tubing. Similar

results were obtained when the experiment was repeated with *Flavobacterium* species.

No bacterial species was isolated from the gases flowing from the anesthesia machine into the circle system.

Discussion

It has long been assumed that the anesthesia machine is a potential source of bacterial contamination to patients.¹² Although several reports suggest that anesthesia machines may have been involved in the transmission of organisms between patients, these reports are for the most part inconclusive.³⁻⁶ Certainly, in view of the large number of anesthetics administered, it is surprising that there is no documented epidemiologic study incriminating the anesthesia machine as the source. Most institutions have regular cleaning procedures, and sterilize equipment that normally comes into direct contact with the patient: endotracheal tubes, masks, corrugated rubber tubing, the "Y" pieces.¹³ As an alternative to decontamination procedures, bacterial filters and disposable equipment have been introduced to minimize the potential hazard of cross-contamination.¹⁴⁻¹⁶ Our studies strongly suggest that, regardless of prior upper airway colonization and duration of anesthesia, patients rarely contaminate the anesthesia machine with significant levels of bacteria. Comparison of the expiratory tubes from colonized patients, expected to be the most contaminated, and the inspiratory tubes from uncolonized patients, expected to be the least contaminated, yielded no significant difference. Examination of this tubing also indicated that microbial contamination inherent in, and added to, it remained stationary along the corrugations, and seemed not to be affected by gas flow or patient expirations. Gram-negative bacilli accounted for only a minute fraction of the contamination of corrugated tubing. The species of bacteria found in corrugated tubing used on colonized patients did not differ significantly from those found in control tubing or from those recovered from the corrugated tubing used on uncolonized patients. The distribution of microorganisms within the tubing could not be correlated with duration of anesthesia, segment position, or extent of prior patient colonization. The number of times each machine is used during a 24-hour period can vary from one to five. This frequently results in a build-up of condensate in the expiratory port, in the expiratory valve leaflet, at the bottom of the CO₂ canister, and along the sides of the two compartments forming the canister. A total of 192 cultures from these areas yielded three positive cul-

tures, two of *Staphylococcus epidermidis* and one of an unidentified gram-negative bacillus. Even after a six and a half-hour upper lobectomy for excision of a *Proteus empyema*, we were unable to demonstrate these organisms in any of the locations cultured. This is further evidence that bacterial migration through the circle system is not likely.

With longer periods of time or greater amounts of bacteria, contamination of the anesthesia machine may possibly occur. Therefore, we attempted to simulate these conditions by deliberately contaminating the expiratory port of an anesthesia machine with a large inoculum of bacteria. We were unable to detect any significant contamination of the CO₂ absorber, moisture at the bottom of the CO₂ absorber, inspiratory port, or inspiratory limb of corrugated tubing following heavy contamination of the expiratory port with two common strains of gram-negative bacilli. That bacteria were disappearing from the original inoculation point over a three-hour period, yet could not be recovered downstream within the system at any time during that three hours, suggests that the organisms were probably being destroyed.

Previous studies have attempted to simulate contamination of anesthesia equipment.^{1,17-20} Most investigators have reported negative findings, and our observations were consistent with these reports. This is not altogether unexpected, since the environment within the circle system is probably not conducive to bacterial survival, for a number of possible reasons, including dessication by a flow of cold, dry, anesthetic gases, bacteriostatic properties of the rubber and metal that compose the circuit, and the highly alkaline condensate at the bottom of the CO₂ absorber over which the gases pass.

Other possible sources of gram-negative bacilli are the hands of the anesthesiologist, the gases added to the circle system, and the ambient levels of bacteria found in the operating room environment. Bacteria were not found in the inlet gases, although in newly constructed pipeline systems particulate contamination may be a hazard to patients.²¹ The hands of the anesthesiologist and the settling plates demonstrated predominantly gram-positive species; thus, it is unlikely that these sources are major contributors of gram-negative bacilli.

In this hospital no attempt is made to clean or sterilize the internal parts of the anesthesia machine. Soda lime is replaced after a known infected patient is handled (although in light of our data this probably is not necessary); otherwise, the machine is re-used until the indicator in the soda lime signals the need for replacement. Sanitary measures should not

be relaxed at this time; however, the optimal combination of cleaning and sterilization remains to be determined. Routine gas sterilization of rubber tubing, bags, "Y" pieces, and endotracheal tubes is more than adequate to provide a reasonable margin of safety. This study does not support the need for use of extra measures such as bacterial filtration. Furthermore, the use of disposable circle systems on the basis of the fear of cross-infection is probably not justified.

Our studies, which examined likely sources of contamination of the anesthesia machine, including colonized and uncolonized patients, the hands of the anesthesiologist, and oxygen and nitrous oxide gas sources, offer strong evidence that the anesthesia machine and circle system do not act as any more of a source of potential infection than other non-invasive equipment. Furthermore, the circle system, even when heavily contaminated with bacteria, does not disseminate bacteria within the system. Consequently, we feel that the anesthesia machine is not a significant fomite in infection. Routine cleaning and sterilization procedures currently in use are sufficient to ensure that the risk of cross-contamination is minimal.

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