SIMPLIFIED GAS STERILIZATION: A NEW ANSWER FOR AN OLD PROBLEM

BY

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SUMMARY

The efficacy of ethylene oxide gas sterilization is well documented. However, the inherent problems of time and expense, the addition of heat and/or pressure have proved to be deterrents in universal adoption of the method. A simplified procedure using 84 per cent ethylene oxide without heat or pressure is presented. Appropriate culture controls were maintained. Once the proper technique was developed, all routine weekly cultures have been negative since November 1966. Since this system requires no maintenance, power source or installation, it is adaptable to almost any environs including catastrophic conditions.

The subject of cleaning anaesthesia equipment has always been a constant source of debate. There are as many divergent opinions on the potential and actual harm to the patient as there are discussions.

Equipment to be sterilized can be considered generally as being of two types: heat-stable and heat-labile. Even the heat-stable article can have its usable life shortened through improper care. Equipment can also be divided into two classes. Critical equipment is that which is in direct contact with the patient, such as endotracheal tubes, mouthpieces or intravenous materials. The non-critical equipment is only indirectly involved, such as breathing bags, breathing circles, ventilators, and related materials.

Contamination can be organic or inorganic. The organic materials can contain bacteria in vegetative or spore form, fungi, parasites, and viruses. All further discussion assumes the basic premise that all articles to be sterilized should be first adequately mechanically cleansed to get rid of gross contamination.

Germicidals can be divided into liquid and gas. The liquid germicidals are shown in the next column.

Among the gas germicidals are those listed on the following page.

In this paper it is intended to discuss ethylene oxide sterilization with particular reference to the Anprolene* system.

*Anprolene is a registered trademark of H. W. Andersen Products, Inc., and consists of 84 per cent ethylene oxide, 16 per cent inert ingredients.
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Gas germicides.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Method of sterilization</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>Coagulation of protein</td>
<td>Corrosive to metals, irritating, works only in humidity greater than 70 per cent</td>
</tr>
<tr>
<td>Beta Propranolactone</td>
<td>Unknown</td>
<td>Carcinogen, unstable, expensive, vesicant</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>Replacement by</td>
<td>Usually needs expensive equipment</td>
</tr>
<tr>
<td></td>
<td>hydroxyl-ethyl radical of sulphhydryl amino, carboxyl or hydroxyl group</td>
<td></td>
</tr>
</tbody>
</table>

and Bossert (1936). Patent number 2,037,439 was issued to Gross and Dixon in 1937 on a "method of sterilization". Later, the process was tried as a food preservative. Even soil was sterilized by this method. In the article by Phillips and Kaye (1949) various methods and aspects of the system were described. Sterli, Reed and Billick (1962) wrote about its use with various hospital items. Snow, Mangiaracine, and Anderson (1962) discussed sterilization of anaesthesia equipment. The list of articles on specific items is endless.

Properties of ethylene oxide.

Ethylene oxide is a cyclic ether compound having the formula

\[ \text{CH}_2 = \text{CH}_2 \quad \text{O} \]

and a molecular weight of 44.05. Liquid ethylene oxide freezes at -111.3°C (168.3°F) and boils at 10.73°C (51.3°F). It will react with water in contact with catalysts such as anhydrous chlorides of iron, tin, or aluminium.

Ethylene oxide is completely soluble in water at room temperature. It will form polyethylene glycol with water in the presence of certain acid and basic catalysts. It is soluble in alcohol, ether, and other solvents, as well as rubber, leather, and plastics.

Liquid ethylene oxide will ignite and burn if directly exposed to flame. Gaseous ethylene oxide in excess of 3 per cent mixed with air is highly explosive and flammable. The source of ignition can be heat, static, open flame, or spark.

Toxicity can be demonstrated in both liquid and gaseous phase. In the liquid phase, it acts as a vesicant on exposed skin. It can cause delayed burns if clothing is worn immediately after exposure to the gas. The vapour is toxic if inhaled and produces irritation of the eyes and mucous membranes. The variability of safety factor is evident by the following recommendations as to safe exposure concentrations.

<table>
<thead>
<tr>
<th>Concentrations of ethylene oxide</th>
<th>Exposure—maximum limit</th>
<th>Source—authority</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 p.p.m.*</td>
<td>8 hours</td>
<td>Manufacturing Chemists Association</td>
</tr>
<tr>
<td>50 p.p.m.</td>
<td>8 hours</td>
<td>Helliingsworth</td>
</tr>
<tr>
<td>50 p.p.m.</td>
<td>8 hours</td>
<td>Linde</td>
</tr>
<tr>
<td>10 p.p.m.</td>
<td>8 hours</td>
<td>Thomas</td>
</tr>
<tr>
<td>5 to 10 per cent</td>
<td>Few minutes</td>
<td>Merck</td>
</tr>
</tbody>
</table>

* No cumulative human toxicity has been reported.

Mode of action.

Sterilization with ethylene oxide is effected by replacement of an available hydrogen atom by a hydroxyl-ethyl radical within a chemical group such as the sulphhydryl, amino, carboxyl, or hydroxyl in the protein molecule. Spore forms are more resistant than vegetative forms. The ease of sterilization in the vegetative form is due to the availability of the sulphhydryl group. Other chemical groups are not as reactive. This is the process of chemical interference, or inactivation of the cell reproductive process.

Requirements for action of ethylene oxide.

Concentration of the gas. The partial pressure of the ethylene oxide is the most important variable. Schley, Hoffman and Phillips (1960) state that a concentration of 400-500 mg/l. is needed for sterilization in 6-16 hours. Phillips (1949) believes that doubling concentration reduces the exposure time by half.

Humidity. Moist bacteria are more susceptible than dried bacteria. Kaye and Phillips (1949) believe relative humidity levels of 20-40 per cent are ideal. The rate of kill is 10 times greater at 28 per cent humidity that at 97 per cent. Mathews and Hofstad (1953) showed that animal viruses are destroyed in the moist but not in the dry...
state. Newman, Colwell and Jameson (1955) showed that tubercle bacilli were killed in moist but not in dried sputum. Opfell, Hohman and Latham (1959) found that bacteria dried on glass or plastic were more difficult to kill than spores on paper or porous material. This is thought to be due to release of moisture on a porous surface. There is also a problem in hydrating spores over a short time, in that exposure to 80-90 per cent humidity for one week causes only 4-5 per cent uptake of moisture.

**Temperature.** The increase in temperature increases the efficiency of ethylene oxide. Heat improves the penetration and so reduces the exposure period. Raising the temperature increases the pressure of the gas within the container.

**Time.** Gas sterilization is not rapid. Time needed is variable, usually 2-12 hours. Spores are 100-1000 times more resistant to killing than the vegetative forms. Lipids in the cells are supposed to be the cause of the resistance. Church and associates (1956) found that extraction of the lipids made bacteria less resistant.

**Limitations of ethylene oxide.**

Liquid ethylene oxide is a solvent, affecting rubber and plastics including lucite and plexiglass. Gaseous ethylene oxide will haemolyze red cells and can inactivate antibiotics such as streptomycin (Kaye, Irminger and Phillips, 1952). Plastic, rubber and leather absorb ethylene oxide and hold it for several hours. Ethylene oxide is usually completely dissipated after 24 hours (Reddish, 1957). Royce and Moore (1955) believe that concentrations as low as 2 mg/g in rubber are liable to cause vesicular lesions. Freeman and Barwell (1960) postulated that the gas in tubing in heart-lung machines could cause bubbles if used immediately after sterilization. It is felt that ethylene oxide in metal-wire-reinforced endotracheal tubes may lead to separation of the latex from the wire. This is probably caused by the increased absorption of ethylene oxide and freon at the elevated temperatures and pressures usually employed followed by the usual vacuum degassing phase.

**Ethylene oxide mixtures.**

The flammability of ethylene oxide can be altered by diluting with inert gases. Coward and Jones (1952) showed that mixtures of ethylene oxide can be made non-flammable by mixing with carbon dioxide. Haenni and associates (1959) describe mixtures of halogens with ethylene oxide. Various combinations are commercially available. The addition of carbon dioxide reduces the cost as well as the toxicity and flammability. The freon-ethylene oxide mixtures have lower vapour pressure and so contain more ethylene oxide per unit volume than a corresponding carbon dioxide mixture at the same pressure. Freon mixtures are also more soluble in materials than mixtures containing carbon dioxide. Freon and other hydrocarbons will cause more adverse effects than carbon dioxide when mixed with ethylene oxide. They are solvents by themselves and will cause etching and other surface phenomena in plastics.

**Preparation and packaging of materials for gas sterilization.**

Materials should be:

1. Permeable to ethylene oxide and moisture. The paper by Dick and Feazel (1960) is concerned with permeability constants and the ease with which the gas will diffuse through various materials.
2. Strong in order to withstand handling and storage.
3. Reasonable in cost.
4. Flexible for ease of wrapping.
5. Impermeable to bacteria.

**Storage of articles after sterilization.**

<table>
<thead>
<tr>
<th>Material</th>
<th>Length of storage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper</td>
<td>3-4 weeks</td>
<td>Tears easily</td>
</tr>
<tr>
<td>Muslin</td>
<td>3-4 weeks</td>
<td>Very porous</td>
</tr>
<tr>
<td>Plastic</td>
<td>Indefinite</td>
<td>1-3 μ (0.001-0.003 in.) limit, low or medium density</td>
</tr>
<tr>
<td>Cellophane</td>
<td>3-4 weeks</td>
<td>Becomes brittle</td>
</tr>
<tr>
<td>Weck</td>
<td>3-4 weeks</td>
<td>Is too variable in thickness and density</td>
</tr>
<tr>
<td>(cellophane tubing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nylon</td>
<td>Indefinite</td>
<td>Best storage characteristic, expensive</td>
</tr>
</tbody>
</table>

**DESCRIPTION OF ANPROLENE STERILIZATION**

For three years the anaesthesia department of the Allentown Hospital has been using a simple portable gas sterilizer. The system consists of a single-use glass ampoule of 84 per cent pure liquid
Ethylene oxide compound with 16 per cent dissolved inert stabilizers in a plastic sheath which is sealed inside a small gas-release bag (fig. 1). When the ampoule is broken, the liquid vaporizes and diffuses out of the gas-release bag into the larger bag into which the materials to be sterilized have been placed. These two bags act as diffusing chambers and allow the gas to remain long enough so that sterilization is accomplished and then safely diffuse the gas. A stainless steel container acts as an open flame and spark shield. No negative pressure is utilized although the sterilizing bag can be squeezed to express excess air. The diffusion of ethylene oxide from the gas-release bag into the sterilizing bag creates a positive displacement of residual air. Also, since the molecular weight of air is approximately 28.8, compared with that of ethylene oxide which is 44, the larger ethylene oxide molecule will create a physical displacement gradient.

This unit is completely portable and requires no power source. Each ampoule releases 3800 mg of ethylene oxide vapour. The molecular weight of ethylene oxide is 44.05 g/mol; therefore, each ampoule releases 0.0863 mol of ethylene oxide. One mol of any volatile releases approximately 22,400 ml of vapour at standard temperatures (0°C) and atmospheric pressure (760 mm) or about 24,000 ml at room temperature (20°C). Therefore each ampoule releases 2075 ml of ethylene oxide gas at room temperature and atmospheric pressure.

After sterilization, items made of non-porous materials, such as surgical instruments, transducers, and strain gauges, can be used immediately. Porous materials such as plastics and conductive rubber are quarantined and averted for 48 hours before use. Using these standards, no instance of a toxic effect has been seen in our experience of some 50,000 cases.
Ethylene oxide is released from the gas-release bag at a rate of approximately 1000 mg/hour for the first 3 hours, after which the rate drops to nil over the next 3 hours. The gas is released from the liner bag at an average rate of approximately 250 mg/hour after the first hour.

Safety can be discussed in an acute and chronic phase. To achieve an acute toxicity of 3000 p.p.m. in a room of 10 x 10 x 10 ft. containing 28,317,000 ml air in a static condition, and assuming no air currents or leaks, 40 ampoules of liquid ethylene oxide would have to be broken and immediately converted to a gaseous state. In this system the exposure would be impossible because the ampoule is within a gas-release bag which slows the diffusion of the gas.

Considering the long-term exposure at the rate of diffusion out of the liner bag, 20 sterilizers would have to be in constant use to exceed 100 p.p.m. or 600 sterilizers in constant use to exceed 3000 p.p.m. One ampoule broken without the bag would yield a concentration of 75 p.p.m. which is below the industrial standard for continuous breathing for an 8-hour day. To obtain an explosive level (30,000 p.p.m.) 400 ampoules without diffusion bags would have to be broken and immediately converted into gas. Six thousand sterilizers would have to be in constant use to reach explosive levels.

**Cultures.**

Cultures were done at both the Allentown Hospital and Temple University to test the effectiveness of the system as well as the wrapping materials (table I). Cultures were the standard Bacillus subtilis (globigii) and Clostridium sporogenes, commonly used to test gas sterilization. After correction of initial problems, all cultures at Allentown Hospital since November 1966 have been negative.

The exposure time was set at 12 hours after preclinical work showed that 4–6 hours was necessary to kill both vegetative and spore bacteria. The extra 6 hours produces a 100 per cent safety factor.

**DISCUSSION**

The wrapping materials tested included paper, glassine, nylon, and plastic. The plastic bags were the various easily available bags manufactured by commercial companies. Thickness and density

<table>
<thead>
<tr>
<th>Glassine</th>
<th>Wet</th>
<th>Positive: 13 (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper</td>
<td>Wet</td>
<td>Positive: 4 (4)</td>
</tr>
<tr>
<td>Paper over paper</td>
<td>Wet</td>
<td>Positive: 6 (2)</td>
</tr>
<tr>
<td>Paper over plastic</td>
<td>Wet</td>
<td>Positive: 1 (4)</td>
</tr>
<tr>
<td>Plastic</td>
<td>Wet</td>
<td>Positive: 1 (4)</td>
</tr>
<tr>
<td>Plastic over paper</td>
<td>Wet</td>
<td>Positive: 7 (1)</td>
</tr>
<tr>
<td>Weck</td>
<td>Wet</td>
<td>Positive: 9 (3)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>Positive: 48</td>
</tr>
</tbody>
</table>

(1) Four cases contaminated through handling, three positive before selected wrapping.
(2) Culture strips were in one bag where indicator tape did not change colour.
(3) Weck tubing is too variable in thickness and density to be acceptable.
(4) Variable supervision on weekends and holidays.

Cultures:
- standard—Clostridium sporogenes, Bacillus subtilis (globigii).
- Culture:
  - dry, no special effort at humidity;
  - wet, deliberate moisture introduced in form of moist pad or dipping.

were ascertained. Most of the readily available "kitchen" bags were of less than 3 μ thickness and of low or medium density. Most heavy gauge original equipment bags were too thick to be considered. Nylon wrappings have good permeability and long shelf life. Weck tubing was used for endotracheal tubes and suction catheters. At the Allentown Hospital no problem occurred and the cultures were negative. At Temple University the same material from a different supplier created a problem in that the sterility was not constant. As a result all Weck tubing at both institutions was discontinued.

Upon appraisal of the positive cultures, a definite trend developed in that the positive cultures occurred most frequently on holiday weeks or weekends when most of the regular personnel were not present. As a result all sterilizer bags are filled at the end of the day shift and the same personnel open the bags the next morning at the beginning of the day shift. In this way responsibility can be achieved and faults rectified promptly. Since in-
constituting this system in November 1966 there has not been a single positive culture at the Allentown Hospital.

No toxicity problems developed. The concentration of the ethylene oxide is high in the beginning but tapers off as the gas diffuses out of the container bag. Therefore, the amount of gas in the bag is small at the end of 12 hours. This is in contrast to other gas sterilizers which maintain a lower concentration at the beginning but finish at the same concentration as they started. No liquid ethylene oxide touches the material to be sterilized because of the barrier provided by the diffusion bag membrane.

The method is economical. After the original spark shield container is purchased, the cost depending on the type, the refill units which contain all the essential materials such as colour break ampoule in a diffusing bag, a bag tie-wire, and the container bag cost a few shillings. There are no maintenance problems or lost time due to gasket failure or power disturbance.

There are two types of bags available having similar volumes but different configuration to fit the two types of stainless steel containers. The limitations of the system are those of gas sterilization and the size of the container. Glass tubing which is closed at one end and thick plastic tubing require two ampoules to achieve penetration. Open drugs and liquids cannot be sterilized due to solubility and chemical reactivity of the ethylene oxide.

The only fault of the system is the lack of positive seal of the container bags. Several times early in the study, unauthorized personnel opened the bags and only an alert member of the department and the use of gas-sensitive tape detected the problem. A seal which could not be closed again would prevent this problem. Equipment constructed of spongy rubber or plastic gas-absorbing material must be quarantined and aerated for a minimum of 24 hours.

We conclude that the Anprolene method of ethylene oxide sterilization is a simple, economical way of sterilizing heat-labile and heat-stable materials. No outside energy source such as heat, water, negative or positive pressure, or electricity is required. Anyone can be taught in a short time to operate it properly. It could be an ideal method in a catastrophic emergency.

REFERENCES


NEW APPARATUS: THE NICHOLSON VENOUS PRESSURE STAND

Sir,—This apparatus as illustrated is designed for use in conjunction with the various commercially available saline venous pressure manometers to measure the peripheral or central venous pressure. It has two main functions. It acts as a stand to hold the venous manometer vertically and also as a sighting device to enable the scale incorporated in the instrument to be aligned with any reference point of the patient acceptable to the clinician concerned.

The instrument is designed to permit alignment by either mechanical (Sykes, 1963) or optical methods (Bethune et al., 1966). Mechanically this is accomplished by the use of a pivoted stainless steel beam, which can swing to a right-angle at either side of the vertical manometer stand, and so placed in this extended position as to be in contact with the reference point of the patient. Optical alignment is provided for use when the patient is inaccessible, and is carried out by the use of a simple gunsight-type device. When not in use a clamp is provided to hold the beam in the vertical position.

The apparatus is manufactured in two models—the “Theatre” and the “Standard”—which, though similar in principle, have several important differences. The “Theatre” model, which is the instrument illustrated, has in addition to the features already described a screw-activated parallel-arm-type movement which permits movement of the apparatus vertically, without displacing the manometer laterally. This mechanism is absent from the “Standard” model which, as it is much lighter, can be moved as a whole by loosening the retaining screw and sliding the instrument up and down the support.

Construction. The construction of both models is mainly of stainless steel. The scale on the venous manometer is marked in black on a white background, and extends from −10 cm to +45 cm. Spring steel clips retain the tubing flat against the scale.

The design is aimed at simplicity of operation and the minimum weight consistent with adequate strength.

The “Theatre” model, which offers certain refinements, is intended, because of its weight, mainly for static installation. The absence of these refinements from the “Standard” model has produced a much lighter instrument which is readily portable and which can also be manufactured inexpensively.

The Nicholson venous pressure stand. In this illustration, the beam is in the measuring position.