ETHYLENE OXIDE TOXICITY

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Ethylene oxide toxicity

A study of tissue reactions to retained ethylene oxide

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Ethylene oxide gas (EO), a common sterilizing agent, readily dissolves in most plastic and rubber equipment. The amount of EO retained in common materials was determined. Polyvinyl chloride tubing absorbed comparatively large amounts of the gas. Natural rubber tubing absorbed lesser amounts than did polyvinyl chloride, and eluted the gas more rapidly. Polyethylene and Teflon both absorbed very little EO. Diffusion curves for all these materials are exponential. As the gas elutes from these materials, it can cause severe burns. The level of retained EO which is toxic to tissues was studied. Polyvinyl chloride and natural rubber tubing containing 0.2 to 19.1 mg. of EO per gram of material and control specimens were implanted subcutaneously in 73 mice. Tissue reactions to EO in polyvinyl chloride and natural rubber were similar, and correlated with the level of retained EO. Levels of EO above 9.0 mg. per gram were always followed by a third degree burn. Levels below 5.6 mg. per gram were never associated with the formation of an eschar. Absorbed EO above 3.5 mg. per gram was always accompanied by a visible reaction. No detectable reaction was found at 2.0 mg. of EO per gram or lower. Aeration for 24 hours of materials exposed to EO in the system studied here provides a generous margin of safety. Polyvinyl chloride tubing which was aired for 6½ hours or longer caused no apparent reaction when implanted. Natural rubber implants showed no reaction after 90 minutes of aeration. Little aeration appears to be necessary for polyethylene and Teflon.

Ethylene oxide (EO), the simplest of the cyclic ethers, now serves as a common sterilizing agent in hospitals throughout the country. Since 1949, when Phillips[1] reported the strong bactericidal activity of EO, numerous studies have

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demonstrated its efficacy as a sterilizing agent.[2-5] Reports have recommended EO for sterilization of a wide range of heat- or moisture-sensitive materials, such as cardiac catheterization equipment,[6] anesthesia equipment,[7] tissue culture media,[8] and arterial grafts.[9] Hence, the use of EO as a medical sterilant has grown tremendously in the last decade.

EO has served as a fumigant and industrial sterilant, however, for 40 years. As such, its toxic potential became apparent. The toxic properties of EO have been reviewed elsewhere.[10-11] Briefly, EO acts as a vesicant, causing burns if confined against the skin.[12-13] The inhalation toxicity of EO is similar to that of ammonia gas.[14] EO has a potent hemolytic effect.[15] Its mutagenic potential is similar to that of radiation.[16]

As EO is extremely soluble, it readily penetrates most porous materials and actually dissolves in some solids such as rubber[17] and plastics.[18] The gas absorbed during the sterilization cycle dissipates after exposure. Complete elution may take several hours or days. Bruch[19] has reviewed the problem of sterilant residues in materials treated with EO.

The increasing use of EO as a medical sterilant requires further understanding of its toxicity when absorbed in materials used on or in the human body. This paper reports observations on the duration of retention of EO in medical materials and the level of EO which is toxic to tissues. A model for the study of retained EO in vivo is presented. Tissue reactions to retained EO in implanted materials are described.

Materials and Methods

Preparation of specimens. Plasticized polyvinyl chloride tubing (5 mm. in external diameter and 3 mm. in internal diameter) with a "piggyback" sump (2 mm. in external diameter and 1 mm. in internal diameter)* was cut into 1 cm. segments weighing 158.4 to 188.3 mg. A flat strip of natural rubber tubing (6 mm. wide and 1 mm. thick)† was cut into segments 2 cm. long, weighing 100.4 to 131.7 mg. Each polyethylene specimen consisted of 2 balls (0.5 cm. in diameter)‡ weighing 185.9 to 190 mg. The Teflon specimens were flat discs (1 cm. in diameter and 1 mm. thick)§ weighing 134.8 to 193.5 mg. Each specimen was wrapped individually in highly porous paper.‖

Sterilization in EO. The specimens were exposed to pure EO¶ at 68 to 82° F. and atmospheric pressure. The sterilizing system used has been described in detail elsewhere.[20] The paper-wrapped specimens were placed in a plastic bag with an ampul containing 3.8 Gm. of EO. The bag was sealed with a twist-tie and placed in a closed stainless steel canister. After 12 hours, the canister and sealed plastic bag were opened. All specimens were removed from the plastic bag and were aired for variable periods.

Determination of residual EO. Each specimen was weighed prior to exposure to EO and immediately before implantation. Many specimens were weighed several times during the airing period. All weighings were performed on a Sartorius Model 2404 Balance.# Whenever possible weighings were done in triplicate and an average weight was used for calculations.

Airing procedure. All specimens were aired in their paper wrappers in a well-ventilated

*Polyvinyl chloride tubing extruded exclusively for H. W. Andersen Products, Inc., Oyster Bay, L. I., N. Y., used as a medical sump tube.
†Natural rubber tubing obtained from Parker-Stearns Rubber Co., Brooklyn, N. Y.
‡Polyethylene balls obtained from Orange Products, Chatham, N. J.
§Teflon discs obtained from Almac Plastics, Inc., New York, N. Y.
‖Top-Towels from Bay West Paper Co., Green Bay, Wisc., were used for wrapping specimens.
¶Anprolene brand E O from H. W. Andersen Products, Inc., Oyster Bay, L. I., N. Y.
#Sartorius Balance obtained from Brinkman Instruments, Inc., Westbury, N. Y.

room. Specimens which were reweighed were replaced in their wrappers after each weighing. Aseptic technique was used in transferring specimens. Control specimens were aired for at least one week prior to implantation.

Mice. Healthy Swiss-Webster male and female mice weighing 25.3 to 48.8 grams were used in these experiments. The mice were seventh and eighth generation descendents of a single litter,* bred by sibling mating.

Preparation of mice. The abdomen was shaved with a cream depilatory,† and cleansed with water.

Implantation. Each mouse was anesthetized with ether. The abdomen was cleansed with 70 per cent alcohol. A 1 cm. scalpel incision was made over the lower sternum and xyphoid. A hemostat was then inserted subcutaneously to form long thin pockets on each side of the abdomen. One control and one test specimen were placed subcutaneously in each mouse. The incision was immediately closed with 2 or 3 interrupted nylon sutures. Polyvinyl chloride specimens were implanted in 44 mice and natural rubber specimens in 29 mice.

Observations. Comparison of the appearance of the test and control sites in each mouse was made within 4 to 6 hours. Further comparison was made at 24, 48, and 72 to 120 hours. The amount of swelling, discoloration, and the presence and size of eschar were recorded. A series of 11 mice, which were implanted with specimens containing 0.2 to 2.8 mg. of EO per gram of polyvinyl chloride or natural rubber were observed "blind." In this series the examiner did not know which side contained the test and which the control specimen.

Histology. The 11 mice followed "blind" were killed by ether anesthesia 48 hours after implantation. The entire implant, including overlying skin and underlying fascia and peritoneum, was excised and placed in formalin. Histologic sections, stained with hemotoxylin and eosin,‡ were examined microscopically. Implants from each mouse were compared. The examiner attempted to determine, by the amount of inflammatory reaction, which was the test and which the control implant.

Results

Absorbtion and elution of EO. Large amounts of EO were absorbed by polyvinyl chloride tubing, but the gas desorbed quickly. Immediately after removal from the sterilizer, polyvinyl chloride specimens contained 27.2 to 28.6 mg. of EO per gram. The concentration of retained EO dropped rapidly in the first 2 hours to an average of 11.2 mg. of EO per gram. Thereafter, the rate of desorbtion slowed. The average retained EO at 6 hours was 3.1 mg. per gram. At 12 hours, 0.7 mg. of EO per gram remained in the material. No detectable EO remained in the polyvinyl chloride specimens at 24 hours. Fig. 1 illustrates the amount of retained EO at various times after exposure. It represents 236 weighings of 85 specimens exposed to EO in 14 separate cycles. The quantity of retained EO varied little from one cycle to the next.

Natural rubber absorbed lesser amounts of EO than did polyvinyl chloride and eluted the gas more rapidly. Initial concentrations of EO were 12.3 to 16.1 mg. per gram. The amount of retained EO averaged 1.3 mg. per gram at 2 hours aeration. All detectable EO eluted from the specimens in 4 to 8 hours. Fig. 2 illustrates the amount of EO retained in natural rubber at various times after exposure. It represents 100 weighings of 44 specimens exposed to EO in 10 separate cycles.

*Original litter obtained from Hemlock Hollow Farms, Paterson, N. J.
†Sleek or Nair.
‡Histologic sections were prepared by the Department of Pathology, Community Hospital, Glen Cove, N. Y.

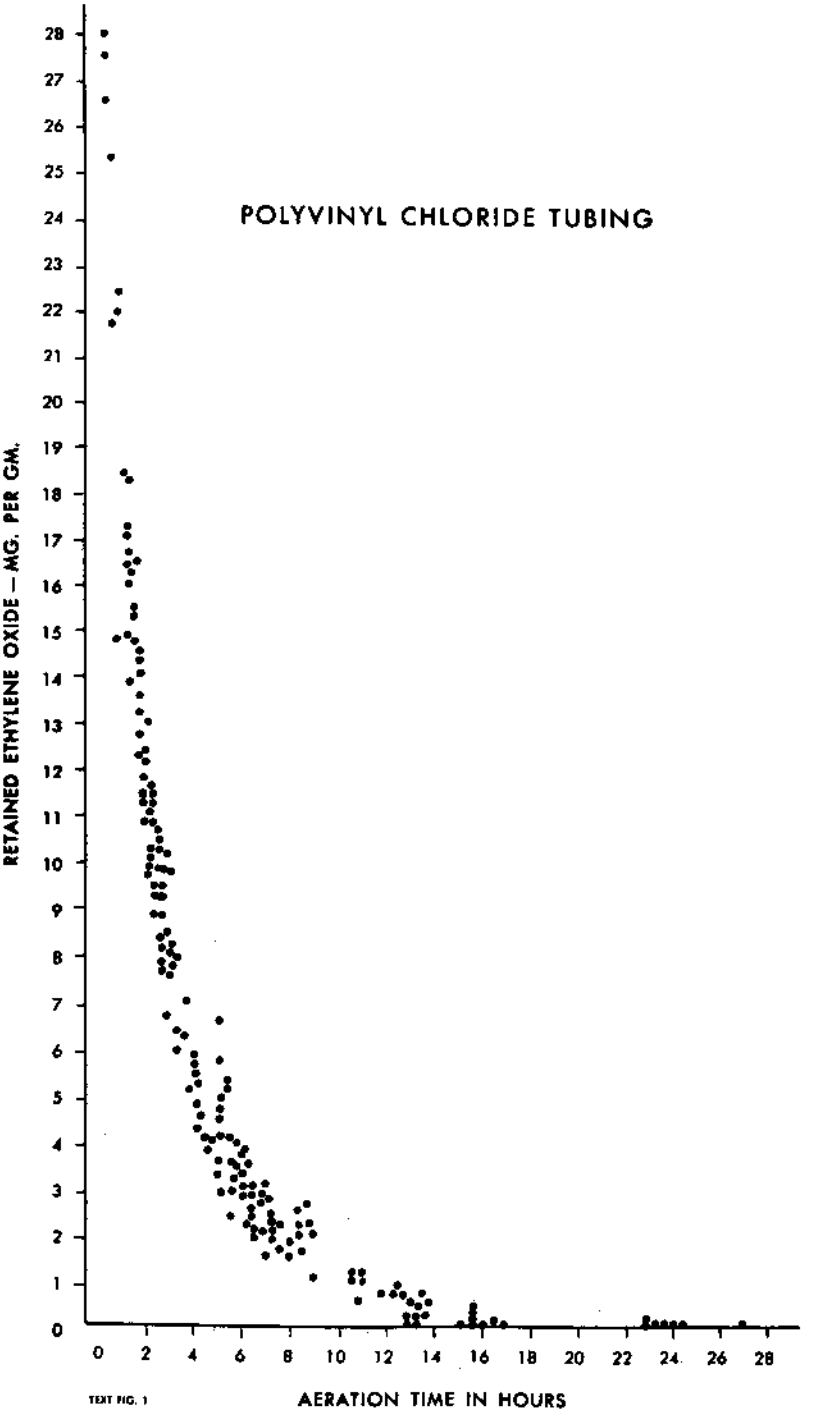

Fig. 1. Desorbtion of EO retained in polyvinyl chloride tubing.

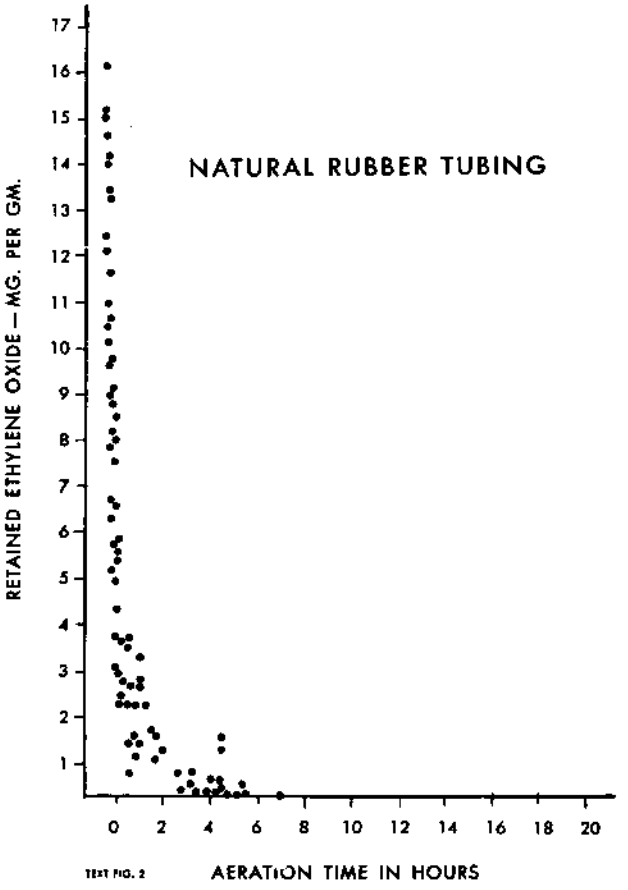

Fig. 2. Desorbtion of EO retained in natural rubber tubing.

Polyethylene and Teflon both absorbed very little EO. The maximum amount of EO taken up by polyethylene was 0.6 mg. per gram. All detectable EO eluted from polyethylene within 5 hours. Similarly, Teflon retained a maximum of 0.6 mg. per gram. No EO remained in the Teflon specimens which were in place from 1½ to 3⅓ hours.

Tissue reactions to implantation of materials retaining EO. Over the 73 control implants, the normal blood vessel pattern was transiently obscured in 7 cases. In 4 of these cases, a trace of edema appeared over the control implant, but the swelling subsided within a few hours. Tissue reactions over the test implantation sites were graded as described below.

SEVERE REACTION. Edema develops very early (within 1 to 2 hours after implantation). Within 3 to 4 hours, the skin over the specimen becomes blanched. Necrosis of the overlying skin is apparent at 12 to 48 hours. An eschar develops between 48 and 72 hours following implantation. The time at which ulceration appears is closely related to the amount of retained EO. Ulceration was apparent

at 12 hours over 5 specimens containing more than 14 mg. of EO per gram of material. When necrosis developed over 19 specimens containing 5.6 to 12.2 mg. of EO per gram of material, it became evident at 48 hours after implantation. The size of the eschar also correlated with the amount of retained EO. The eschars which developed over specimens containing less than 10.0 mg. of EO per gram were invariably small. Higher EO doses were associated with more extensive eschars.

MODERATE REACTION. Edema develops early, in this instance, but not as immediately as in the severe reaction. Swelling of the tissues over the specimen is noted in 2 to 5 hours after implantation. At 24 hours, the skin over the specimen is edematous and pallid. This induration gradually subsides in 72 to 96 hours. All traces of reaction disappear within 6 days. These changes were observed over 13 implants containing from 5.2 to 8.6 mg. of EO per gram of material.

MILD REACTION. Slight edema over the implant appears 5 to 6 hours after implantation. Slight pallor of the skin over the specimen is sometimes seen at the time of maximal edema (12 to 24 hours). The edema disappears completely within 31 to 48 hours, at which time no difference is apparent between test and control sites. This reaction was observed over 15 implants containing between 2.0 and 7.2 mg. of EO per gram of material.

NO REACTION. No difference between test and control sites was found over 10 specimens containing less than 2.0 mg. of EO per gram. Eleven specimens containing between 2.0 and 3.3 mg. of EO per gram of material also showed no reaction.

The series of 6 polyvinyl chloride implants which were observed "blind," contained between 2.8 and 0.2 mg. of EO per gram. No difference was found between test and control sites in this group. The 5 natural rubber implants which were observed "blind" contained between 2.8 and 0.5 mg. of EO per gram. Of this group, only the specimen containing 2.8 mg. EO per gram showed a mild reaction. No reaction was observed over rubber specimens containing between 2.0 and 0.5 mg. per gram. Histologic sections of these specimens failed to help differentiate the 11 test and 11 control specimens. Notable differences were apparent in the degree of reaction of individual mice. Those animals which demonstrated greater histologic evidence of inflammation around the implants reacted equally to both test and control specimens. Those animals which showed little reaction did so in both test and control specimens.

Fig. 3 illustrates the tissue reactions observed over polyvinyl chloride test specimens. The reactions over natural rubber specimens are illustrated in Fig. 4.

Discussion

These experiments show that EO dissolved in polyvinyl chloride, natural rubber, polyethylene, or Teflon diffuses from these materials at a reproducible rate. Diffusion curves for these materials are exponential. The amount of retained EO in polyvinyl chloride is negligible within 16 hours. No detectable EO remains in natural rubber after 8 hours. All detectable EO eluted from polyethylene within 5 hours and from Teflon within 3½ hours. Initial data on the elution

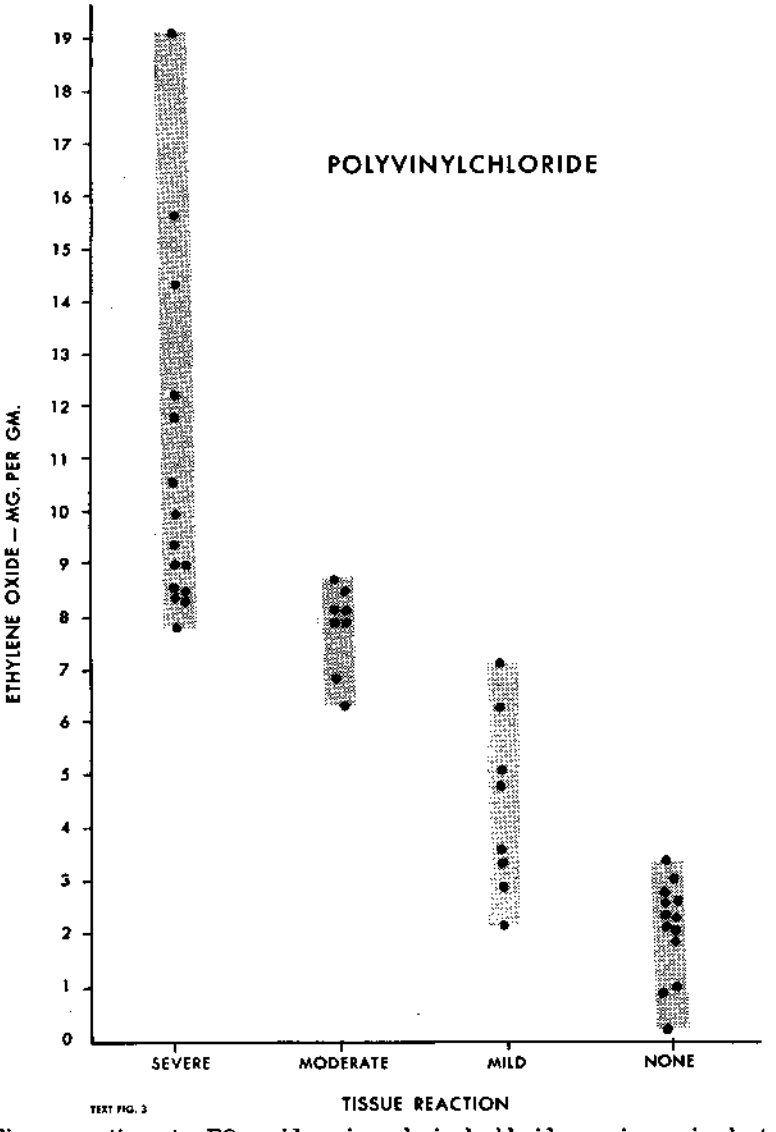

Fig. 3. Tissue reactions to EO residues in polyvinyl chloride specimens implanted subcutaneously in mice.

of EO from other materials such as silicone rubber and polystyrene also reveals exponential curves. Each material shows an individual desorbtion curve which is completely reproducible.

In these experiments, the amount of EO retained was calculated by weight. The balance used for all weighings is accurate to the fifth decimal place. This method of determining residual EO appears to be dependable when a pure EO, ambient humidity system is used. Gas chromatographic determinations of re-

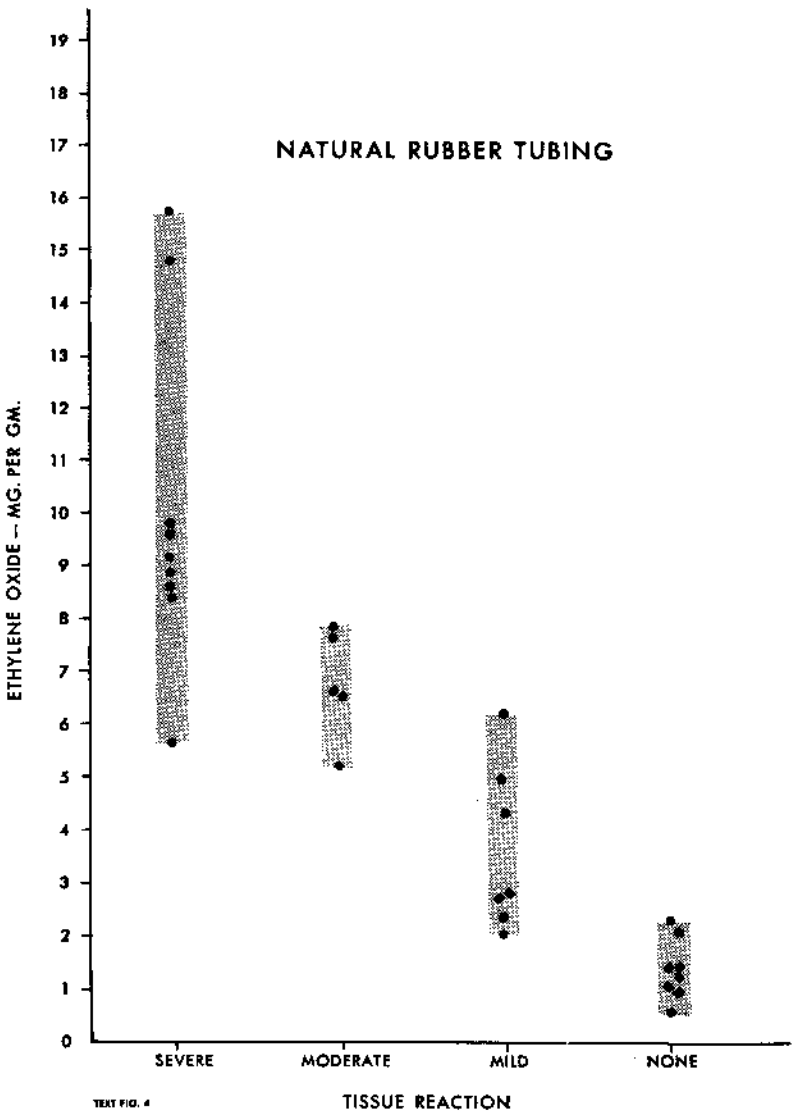

Fig. 4. Tissue reactions to EO residues in natural rubber specimens implanted subcutaneously in mice.

tained EO in natural rubber, performed for comparison, showed comparable results.*

The elution curves recorded here are consistent with the data reported by others.[21-22] Lloyd and Krahe[23] found that EO retained in rubber tubing dissipated almost completely within 5 hours. Residual EO in Tygon tubing was negligible within 19 hours.

*Personal communication: F. R. Kronenwett, Ph.D., American Biological Control Laboratories, Inc., Tenafly, N. J.

A number of factors can affect the desorbtion of EO. Materials used for wrapping may markedly alter EO diffusion. In these experiments, all materials were wrapped in highly porous paper, through which EO appeared to diffuse readily. Paper of low porosity, coated paper, or plastic wrappers may affect both absorbtion and elution of EO.[24] This factor may contribute to the differences between the elution curves recorded here and those reported elsewhere.[25, 26]

The system of EO sterilization employed may also be expected to affect absorbtion and elution of the gas. In these experiments, materials were sterilized at room temperature, under atmospheric pressure, at ambiant humidity with pure EO. The system employs a 12 hour cycle in which gradual release of EO from a plastic gas-diffusion membrane provides maximum concentrations of the gas of approximately 800 mg. per liter at 3 hours. Thereafter, the concentration of EO in the canister and in exposed materials falls steadily. Sterilizing systems which expose materials to a mixture of hot gases (eg., EO and freon) under pressure may be associated with higher initial levels of retained EO. The desorbtion period required for complete elution for such systems may be longer. Reports state that complete elution of EO from polyvinyl chloride, after exposure to EO mixed with carbon dioxide or freon, takes 100 or more hours.[27, 28]

The ratio of the surface area of an exposed object to the total mass of material in the object also affects elution time. Retention of EO in a polyvinyl chloride plug was determined for comparison with polyvinyl chloride tubing. Although the initial concentrations of retained EO were comparable, the plug required a significantly longer period for complete elution.

The in vivo experiments described here are designed to study the potential toxicity of EO retained in medical equipment. The materials chosen for implantation are widely used in endotracheal and nasogastric tubes, wound drains, arterial cannulas, and urinary catheters. In these experiments, the retained EO diffuses through the tissues surrounding the implants. The severity of the resulting tissue reaction correlated well with the amount of retained EO, determined by weight. Retained EO in excess of 9.0 mg. per gram of material was always associated with a third degree burn. Retained EO below 5.6 mg. per gram was never followed by the formation of an eschar. Levels of retained EO above 3.5 mg. per gram were always associated with a visible reaction. No detectable reaction was found at 2.0 mg. of EO per gram of material or lower.

Little other information is available concerning the level of retained EO which is toxic to tissues. The few comparable reports available, however, demonstrate a similar level of toxicity. Royce and Moore[21] found that an occupational dermatitis resulted from exposure to rubber gloves aired 30 to 90 minutes, retaining 3 or more mg. of EO per gram of material. The dermatitis was prevented by airing the gloves for 2 hours before use, reducing the level of retained EO to 2.0 mg. per gram or less.

Matsumoto and associates[25] found increased hemolysis of blood incubated in segments of rubber catheters exposed to EO and aired for 24 hours, when the retained EO was 2.4 mg. per gram. After 48 hours aeration, when the retained gas was 0.09 mg. per gram, no increase in hemolysis was noted. Results of sub-

cutaneous implantation of plastic and rubber tubing exposed to EO paralelled those of the hematologic study.

Other reports demonstrated increased hemolysis of blood incubated in tubing sterilized 24 to 48 hours earlier in EO. However, incubation of the blood for 24 hours was necessary to demonstrate this effect. The amount of retained EO was not determined.[15, 29] The hemolytic potential of certain plastics is striking.[30]

In the system studied here, 24 hours aeration would provide a generous margin of safety. No tissue reaction was apparent to implanted polyvinyl chloride tubing which had been aired for 6½ hours or longer after exposure to EO. Natural rubber implants showed no reaction after 90 minutes aeration or longer. Little aeration seems to be necessary for polyethylene and Teflon sterilized by this system.

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