THE BACTERICIDAL ACTION OF PROPYLENE GLYCOL VAPOR ON MICROORGANISMS SUSPENDED IN AIR. I

By O. H. ROBERTSON, M.D., EDWARD BIGG, M.D., THEODORE T. PUCK, Ph.D., and BENJAMIN F. MILLER, M.D.

With the Technical Assistance of Elizabeth A. Appell

(From the Department of Medicine, the Douglas Smith Foundation for Medical Research, the Bartlett Memorial Fund, and the Zoller Memorial Dental Clinic of the University of Chicago, Chicago)

Plates 18 and 19

(Received for publication, February 27, 1942)

The idea of employing bactericidal mists as a method for controlling airborne respiratory infection is not new, but until recently no one had succeeded in producing by such means a sterile or relatively bacteria-free atmosphere which could be tolerated by human beings. During the past few years new methods of chemical air sterilization have been devised. These consist in the dispersal of germicidal mists containing the effective chemical agents in such small amounts as to be non-detectable or at least unobjectionable to persons in the treated atmosphere. The compounds employed for this purpose are believed to be non-toxic in the minute amounts present in the inspired air.

The initial report on this new approach to the control of air contamination was made by Douglas, Hill, and Smith (1) in 1928. By means of a very fine spray of electrolyzed sea water, containing NaOCl with about 1 per cent available chlorine, these workers were able to effect a marked or complete killing of *Bacillus coli* dispersed in the air. The material appeared to be non-irritating in the concentration employed, which was approximately 1 gm. of the chemical solution in two million cc. of air. This paper apparently attracted little attention and it was not until 10 years later that active development of the subject began.

In 1938 two publications appeared, one by Trillat (2) concerning the properties of germicidal aerosols, and the other by Masterman (3) on air sterilization by spraying or atomizing hypochlorite solutions. Trillat's earlier investigation, covering a period of many years, dealt with problems of droplet infection and the various properties of aerosols, and culminated in his discovery of the sterilizing properties of germicidal aerosols.[1] Trillat found that certain germicidal agents which killed bacteria in the test tube in dilutions not higher than 1:200, were capable of causing death of airborne bacteria when dispersed in aerosol form in concentrations of 1 gm. of the chemical substance in 5,000,000 cc. of air. He believed that this bactericidal activity was due to direct interaction between the aerosol droplets and the bacterial particles.

[1] Liquid aerosols consist of droplets 1 to 2μ in diameter, dispersed in air. An erroneous use of the term aerosol has been introduced by commercial concerns who have applied it as a trade name to certain wetting and detergent compounds.

Trillat tested a number of common bactericidal compounds and found that of these only resorcinol and sodium hypochlorite were satisfactory. The other compounds were either inactive as aerosols or too toxic, or proved to be irritating or unpleasant. He states that resorcinol is the agent of choice as the odor of hypochlorite becomes disagreeable after a time.

Masterman presents evidence for the germicidal effect of fine mists of sodium hypochlorite. He found that 1 gm. of 1 per cent NaOCl atomized in as large a volume as forty million cc. of air would produce sterilization. Masterman concludes that this marked bactericidal action is due to the HOCl gas liberated from the mist and not to an aerosol effect (3, 4).

During 1939 and 1940, two groups of workers in England, Pulvertaft and Walker (5), and Twort, Baker, Finn, and Powell (6), confirmed and extended Trillat's and Masterman's observations. Pulvertaft and Walker tested various substances for activity as germicidal aerosols and recommended a solution of resorcinol in glycerol and water as satisfactory. These workers also found that NaOCl was highly effective and killed air-borne bacteria in a dilution of 1 gm. of 2 per cent NaOCl in six million cc. of air. Their test microorganisms included pathogenic invaders of the respiratory tract as well as non-pathogens. Twort (6) and associates carried out an extensive investigation of the physical properties of aerosols, their droplet size and rate of evaporation, and the effects of various germicidal agents on a number of different microorganisms under a variety of conditions. Their most effective aerosol solution "S^2" contained 10 per cent hexylresorcinol and 0.05 per cent alkyl sulfate, "lorol," in alkaline propylene glycol. They reported bactericidal effects on certain non-pathogenic microorganisms with extraordinarily small amounts of this material, *e.g.* 1 gm. to four billion cc. of air.

Andrewes and coworkers (7) published a brief confirmatory report on the use of bactericidal mists for air sterilization, and in addition noted that a few viruses, including that of influenza, are susceptible to the mist action as judged by the reduction of their infectivity for mice.

Twort and Baker (8) have proposed another and quite different type of agent for air sterilization, namely, certain kinds of smokes. Smokes from ignited cardboard soaked with potassium nitrate or from incense were found to be highly effective. They report that 1 gm. of the chemical substance dispersed in smoke form in 500 million cc. of air causes destruction of 95 per cent of air-suspended bacteria within 15 minutes.

Our earlier work in this field consisted in an investigation of the air-sterilizing activity of certain bactericidal substances used as aerosols (9). We first employed certain of the synthetic detergents studied by Miller and Baker (10), since their activity *in vitro* gave promise of greater effectiveness as bactericidal, aerosols than the compounds used by the French and English workers. Preliminary experiments indicated only moderate effectiveness of aqueous solutions of these detergents. However, when the water was replaced by a hygroscopic vehicle such as propylene glycol, the aerosol activity was markedly

increased. We found subsequently that propylene glycol and certain related glycols themselves act as effective bactericidal aerosols.[2]

As the work progressed it was found that propylene glycol in vapor form was highly bactericidal, and that the marked and rapid germicidal action of propylene glycol aerosol was due to vapor liberated from the small glycol droplets. When pure vapor was employed, it was found to be more effective (12) than an equal quantity of propylene glycol dispersed as an aerosol. For the purpose of explicit exposition, the work will be presented in the sequence of its development.

Methods and Materials

Bacterial Suspensions.—Standardized bacterial suspensions were made by resuspending the centrifugated sediment of an actively growing culture in nutrient broth to a predetermined opacity corresponding to approximately one billion microorganisms per cc. Water and other diluents were employed occasionally. The suspensions were sprayed into the chamber with a Graeser atomizer (14). In some experiments other atomizers producing coarser droplets were used.

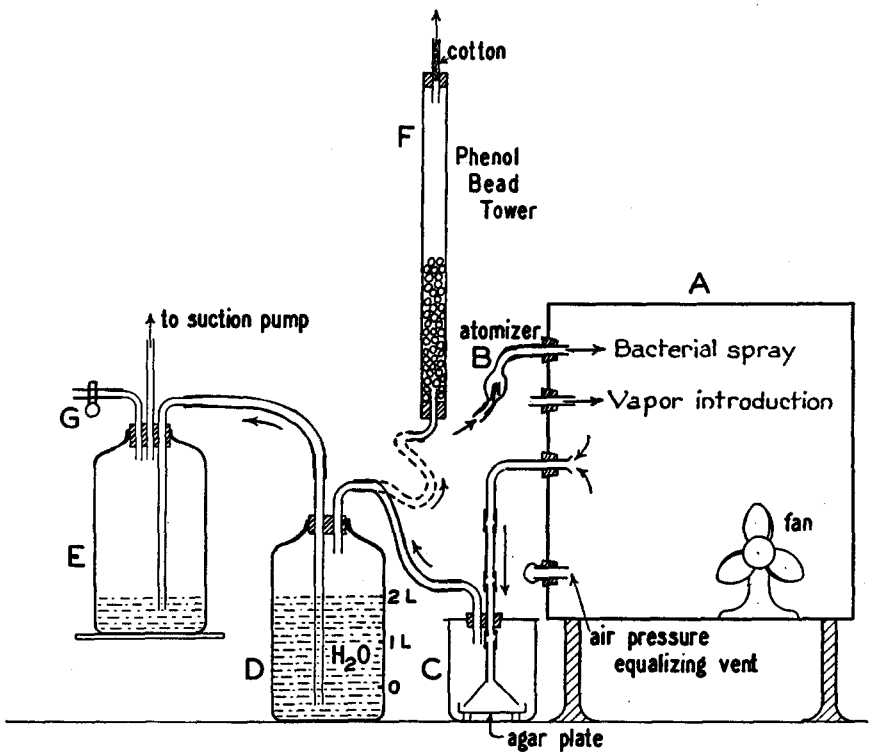
Media for Recovering Bacteria from Air.—For *Staphylococcus albus* and most other non-pathogens nutrient agar with 0.5 per cent added dextrose was used. Rabbit blood agar was employed for recovering pneumococci and *Streptococcus viridans*; sheep blood agar for hemolytic streptococci and staphylococci and chocolate agar for *Hemophilus influenzae*. It was found that chilling the agar plates before use resulted in maximum recovery of bacteria from the air samples.

Test Chambers.—The chambers were made of glass and metal as shown and described in Text-fig. 1. Wooden chambers were found to be somewhat less satisfactory. The air was gently agitated by means of a small fan rotating at a rate sufficient to produce detectable air movement in all parts of the chambers. A metal fan with 4 blades each 2½ inches long, run at 75 volts by means of a variable transformer which gave a speed of 500 R.P.M., proved satisfactory for this purpose. The fan was run for a period of only 5 minutes after the introduction of the bacterial spray.

Method of Sampling Air.—The number of viable bacteria recoverable from the chamber air was determined by withdrawing a measured volume of air at a constant

[2] Although the English workers used glycols and glycerin as vehicles, they apparently ascribed little or no importance to these compounds beyond their usefulness as hygroscopic solvents for the germicidal substances, resorcinol and hexylresorcinol. The only reference to a possible independent action of propylene glycol is that made by Twort and coworkers (6), who reported a single experiment in which solutions of propylene glycol in alcohol exerted a very high degree of bactericidal activity when dispersed in mist form. It seems probable, however, that the germicidal action of this mixture was principally due to the alcohol, since alcohol vapor itself possesses marked germicidal properties. Furthermore, Baker and Twort state in a recent paper (13) that the presence of propylene glycol in their S^2 mixture contributes little or nothing to its bactericidal activity.

rate (2 liters in 2 minutes) through a glass funnel which was suspended directly above the surface of an agar plate within a sealed glass jar (Text-fig. 1). This is a modification of the technique of air sampling described by Hollaender and DallaValle (15) The rubber and glass connections are made as short as possible. The air vent for equalizing internal and external pressure is covered with one or two layers of canton



TEXT-FIG. 1. Apparatus employed for determining bactericidal activity of glycol vapors. *A*, 60 liter air-tight glass-walled chamber with one metal wall (opposite the side with vents) fitted with a door closing on rubber gaskets. (All sides, 15 inches square.) The three upper orifices are actually on a horizontal line across the center of the wall. *B*, Graeser atomizer connected with inlet by rubber tube. *C*, sampling jar. *G*, air vent allowing water to return from bottle *E* to *D*.

flannel. When pathogenic microorganisms were being studied, the orifices of the sampling jars were clamped and the jar containing the agar plate placed in the incubator. Before opening, a considerable volume of air was drawn through the jar and passed through a bead tower containing phenol (Text-fig. 1). Likewise, after each sampling the air from the measuring bottle was expelled through phenol in the bead tower. At the end of experiments with pathogens, the chambers were filled with propylene glycol aerosol or vapor and this air drawn through the suction bottles. Canton flannel masks were worn as an added precaution.

Propylene Glycol.—The propylene glycol used in this study[3] was clear, colorless, and odorless, and when fractionated at a pressure of 25 mm., more than 80 per cent of the material was collected at a temperature of 99–101°C. (The boiling point given in the literature is 100–101.8°C. at 24 mm.) Propylene glycol is relatively non-volatile—its normal boiling point is 188°C., and at 20°C. its vapor pressure is 0.18 mm. Hg. Thus a concentration of 0.73 mg. per liter in the vapor state represents atmospheric saturation. No difference in behavior was detectable when the stock propylene glycol or the redistilled material was used in the experiments described in this paper.

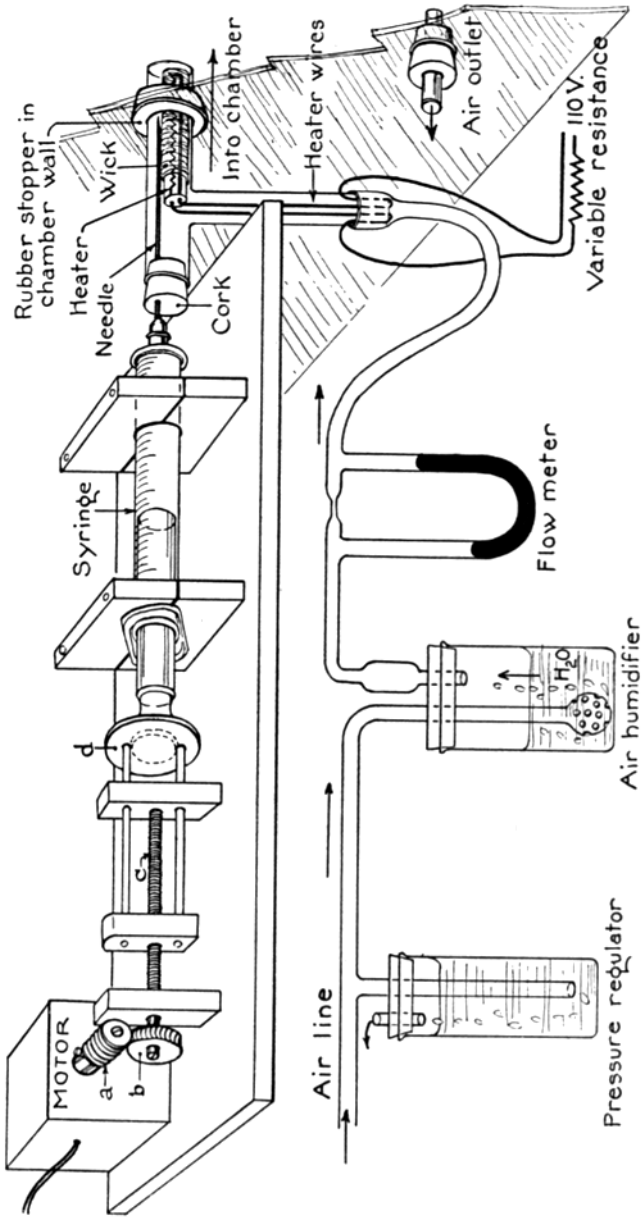
Production of Propylene Glycol Aerosol.—An effective aerosol was produced by means of the Graeser atomizer which delivers a dry mist consisting of droplets averaging 2 to 3μ in diameter. The DeVilbis atomizer No. 180 is also satisfactory.[4]

Production of Propylene Glycol Vapor.—The preparation of atmospheres containing various concentrations of propylene glycol in the vapor state was accomplished in a number of different ways. Placing the glycol in three Petri dishes on the floor of the chambers, and allowing it to evaporate overnight (for 14 to 16 hours) results in a vapor concentration of 0.35 mg. per liter, a quantity sufficient to produce almost immediate killing of the various organisms tested in this study. More rapid and complete saturation is effected by pouring propylene glycol, heated to 70–80°C., into three Petri dishes placed in the chamber. The chamber is sealed, and allowed to cool to room temperature while a fan circulates the air inside. Still another method used consisted in filling the chamber with air bubbled through propylene glycol heated to 60°C. (in a thermostated water bath), which resulted in a glycol concentration of 0.66 mg. per liter in the vapor state.

In order to achieve a more rapid and accurate means of filling the chambers with any desired concentrations of propylene glycol, the apparatus shown in Text-fig. 2 was constructed. A 60 cycle synchronous motor of 1 R.P.M. speed drives forward a screw (*c*) of 20 threads per inch by means of a worm drive (*a*) and gear (*b*), (ratio 7½:1) causing plate (*d*) to move forward and advance the plunger of a syringe. Propylene glycol contained in the syringe is forced out at a constant rate on to a heater which volatilizes the material. The heater consists of a 75 ohm, 10 watt "ohmite" resistor, across which 30 volts are applied. The needle of the syringe rests on a wick made up of a strip of cotton tape wound around the porcelain covering of the resistor. The wick serves to disperse the liquid over the hot surface. An air stream whose rate of flow is controlled by a pressure regulator and measured by a calibrated flow meter, impinges on the heater and carries the volatilized glycol into the chamber. The glass T tube (inner diameter 15 mm.) in which the heater is contained must be as short as possible to obviate the possibility of condensation of the glycol on the walls before it has been adequately mixed with the incoming air stream. Humidity is controlled by a humidifier placed in the path of the air stream, as shown in Text-

[3] We are indebted to the Carbon and Carbide Co. for supplying us with this highly purified material. It should be pointed out that there are preparations of propylene glycol on the market which contain impurities in toxic amounts.

[4] We wish to express our appreciation to the DeVilbis Co. for making a special experimental atomizer for us.



TEXT-FIG. 2. Apparatus for the introduction of air containing calculated concentrations of glycol vapor.

fig. 2. The relative humidity can be varied either by changing the temperature or the amount of the water in the humidifier. Relative humidity inside the chamber was measured by means of wet and dry bulb thermometers, with the wet bulb placed opposite the fan.

The concentration of propylene glycol vapor in the mixture issuing from the T tube is readily calculated, since both the rate of delivery of liquid propylene glycol and the rate of air flow through the vaporizer are known. Any desired concentration may be secured either by changing the rate of flow of air through the flow meter, or by inserting a syringe of a different diameter in the syringe holder. One cc. and 2 cc. syringes were found to be most suitable.

The gas stream fed into the chamber is mixed completely with the air already present by means of a rapidly rotating electric fan and then passes out through an escape vent. A volume of gas four times that of the chamber is swept through for each filling. (Calculation shows that after four air changes under these conditions the propylene glycol content of the gas inside the chamber is 99.0 per cent of that in the entering air stream.) The chamber is then sealed off. Before beginning the experiment a sample of air is removed for analysis as an additional check on the glycol concentration.

Method of Determining Concentration of Propylene Glycol Vapor in Air.—The determination of the concentration of propylene glycol vapor was accomplished by withdrawing a 2 liter sample of the air and bubbling it through 10 cc. of water with the aid of a fairly porous, fritted glass gas-disperser. Complete absorption of the propylene glycol is obtained at a sampling rate of 1/5 liter of air per minute.

The propylene glycol content of the resulting solution can be analyzed by the method of Lehman and Newman (16), modified to accomodate the smaller concentrations involved (17). In this reaction the propylene glycol is quantitatively oxidized by a standard amount of periodic acid. The remainder of the oxidizing agent is determined by reduction with a known quantity of sodium arsenite, and the excess arsenite titrated with I_2. The contents of the test tube are quantitatively washed into an Erlenmeyer flask. 1.0 cc. of M/10 periodic acid[5] is added, and the sample is placed in an ice box for 15 minutes. At the end of that time, 5 cc. of 7 per cent $NaHCO_3$ is added, then 2.5 cc. of N/10 Na_3AsO_3, followed by 0.4 cc. of freshly prepared 20 per cent KI. The solution is allowed to stand for 15 minutes at room temperature, after which 1 cc. of 1 per cent starch is added. The solution is then titrated with 0.01 N I_2 to the end point marked by the appearance of the blue color of the starch iodine complex. A blank is run through the same procedure, and the number of milliliters of I_2 solution used in the blank is subtracted from that required by the sample. One cc. of 0.01 N I_2 solution is equivalent to 0.38 mg. of propylene glycol. Samples containing known amounts of propylene glycol varying from 0.3 to 1.0 mg. gave results accurate to within 0.04 mg., when analyzed by this method.

[5] The M/10 periodic acid is prepared by dissolving 5.35 gm. of sodium periodate ($NaIO_4$) in 75 cc. of N/1 sulfuric acid, and diluting to 250 cc. in a volumetric flask. The resulting solution is stable indefinitely if stored in an ice box between runs. Directions for preparation of the standard sodium arsenate and iodine solutions are available in any standard textbook such as Pierce, W. C., and Haenisch, E. L., Quantitative analysis, New York, John Wiley & Sons, 1937.

EXPERIMENTAL

Reproduceability of Bacterial Counts in Air Samples.—Agar plates exposed to the flow of bacteria-containing air by means of the technique outlined above yielded a remarkably uniform distribution of bacterial colonies. Even though the bacteria recovered on the agar surface represented only a very small per cent of the numbers sprayed into the chamber, the results of sampling were relatively constant. Table I gives the results of air samples taken from two chambers following the introduction of the same amount of standardized suspension of *Staphylococcus albus.*

TABLE I

Control on Method of Taking Samples from Enclosed Atmospheres into Which the Same Amount of Bacterial Suspension Has Been Sprayed

Dilution of standard suspension and time sprayed	Suspension No.	Test No.	Chamber No.	Colonies on plate samples taken	
				Immediately	After 5 min.
1:10 for 30 sec.	1	1	III	364	237
		2	IV	343	283
		3	IV	312	266
	2	1	III	328	250
		2	IV	311	242
1:5 for 45 sec.	3	1	III	1154	912
		2	IV	1254	878
	4	1	IV	910	658
		2	III	922	566
		3	IV	948	636

Two different standard suspensions of staphylococcus were made up from the same culture on each of two occasions. The dilutions of the standard suspensions and the time of spraying are indicated in the first column of the table. The same atomizer filled to the same level and operated at 500 mm. air pressure was used in all the tests. Air samples (of 2 liters each) were withdrawn at exactly the same intervals following termination of the bacterial spray. The first sample (labeled immediately) was started 15 seconds afterwards and the second one at 5 minutes. After two samples had been taken, the chamber was cleaned out and the next test run under identical conditions.

It will be noted in the table that suspensions 1 and 2 yielded approximately the same number of colonies on the plates while the difference in sampling yields between suspensions 3 and 4 indicates that we were less successful in making up suspensions of equal density.

There is a progressive and quite constant diminution in the number of colonies recovered from successive air samples during the period of an hour, as illustrated in Plate 18, Figs. 1 to 4. This phenomenon may be ascribed to settling of the bacterial droplets, inelastic collisions with the chamber walls, and natural mortality of the bacteria. This necessitated the use of two identical chambers for each experiment, one for the test, the other as a control.

Propylene Glycol Aerosol

Tests on the bactericidal activity of propylene glycol aerosol were performed as follows: usually the bacteria were sprayed into the chamber first, and an air sample obtained, following which a weighed quantity of the aerosol was introduced. In order to control any possible effect of the germicidal aerosol other than its direct bactericidal action, distilled water was sprayed into the control chamber in each experiment. It was found that a concentration of

TABLE II

Effect of Propylene Glycol Aerosol on Staphylococcus albus

Aerosol concentration	Time intervals of air samples	No. of colonies on plates from	
		Control chamber	Test chamber
1:2,000,000	Immediately after bacterial spray	2140	2124
	Immediately after H_2O in control and aerosol in test	1940	3
	15 min. later	510	0
	30 min. later	350	0

1 gm. of propylene glycol aerosol in two million cc. of air effected complete sterilization of an atmosphere into which as many as 500,000 bacteria per liter of air had been sprayed. Furthermore, this action occurred with surprising rapidity. Air samples taken within a few seconds after the introduction of the aerosol yielded sterile plates while similar plates from the control chamber showed many hundreds or thousands of colonies depending on the amount of bacterial suspension used. A protocol of an experiment with *Staphylococcus albus* is recorded in Table II and photographs of another experiment are shown in Plate 18, Figs. 1 to 8.

A number of other microorganisms were found to be similarly susceptible to the action of this aerosol. Among those tested were pathogenic invaders of the respiratory tract, *i.e.*, pneumococcus Types I and III, hemolytic streptococci, and hemolytic staphylococci, as well as organisms of lesser or no pathogenicity, such as *Streptococcus viridans*, *Bacillus coli*, *Micrococcus catarrhalis*, and *Bacillus subtilis* (vegetative form).

The order of introduction of the bacteria and the propylene glycol into the

chamber was found to have no effect on the result. Thus an equally rapid sterilization was obtained when bacteria were introduced into a test chamber already containing propylene glycol. Other glycols, such as ethylene and trimethylene, acted about as effectively as propylene glycol. Glycerin, on the other hand, exhibited only a slight killing effect.

Propylene Glycol Vapor

(*a*) *Bactericidal Effect of Aerosol Not Explainable on Basis of Droplet Interaction.*—If the disappearance of bacteria in air treated with a propylene glycol mist represents a true bactericidal effect, and evidence presented later indicates that such is the case, how can this action be explained? The means by which bactericidal mists produce a lethal concentration of the active agent in the immediate environment of the bacteria would seem to be limited to two possibilities; (*a*) direct contact between germicidal aerosol droplets and bacterial particles; (*b*) production of sufficient vapor or gas by evaporation from the germicidal droplets to permit rapid and abundant collision of gas molecules with the bacterial particles. Trillat, Pulvertaft and Walker, and Twort and his associates, believe that germicidal mists exert their antibacterial action exclusively as aerosols. They state that the substances employed by them are ineffective in the gas phase. In fact, their investigations of different agents or mixtures for use as bactericidal aerosols have been directed toward developing a mist with a very slow rate of evaporation. Masterman, on the other hand, considers that the activity of the germicidal mist he employed, namely NaOCl, is due to the liberation of HOCl gas.

Calculations of the maximum number of contacts possible between aerosol and bacterial droplets (which Twort *et al.* have made (6) and which we have repeated) indicate that it would take between 2 and 200 hours for sterilization to occur if this were the mode of action of the germicidal aerosol. Since complete sterilization of a heavily contaminated atmosphere has been found to take place in as short a time as 5 minutes by the English workers, and within a matter of seconds by ourselves, the rate of interaction between the bactericidal agent and air-suspended bacteria must be of an entirely different order of magnitude. Such rapid interaction could occur only if the germicidal substance were present in the gas phase.

(*b*) *Postulation of Vapor-Droplet Effect.*—Granted that rapid interaction between gas molecules and droplets does occur, would the resulting concentration of propylene glycol in the bacterial droplets be sufficient to produce the striking bactericidal effects observed? Experiments *in vitro* showed that propylene glycol in common with other closely related glycols exhibited relatively low germicidal action. Certain microorganisms grow well in broth containing as much as 5 to 15 per cent of the different glycols tested (propylene, ethylene, and trimethylene). The pneumococcus, for example, grows in

broth containing up to but not more than 5 per cent propylene glycol. *Staphylococcus albus* is not inhibited until a concentration of more than 10 per cent of this material is reached. However, when these bacteria are suspended in 80 to 90 per cent propylene glycol, they are killed immediately. Since propylene glycol possesses a marked affinity for water, rapid absorption of the vapor by aqueous droplets might be expected to occur. Indeed, calculations show that with vapor concentrations even considerably below the saturation value of propylene glycol, the number of collisions between gas molecules and droplets containing bacteria is sufficient to produce almost instantly, a lethal concentration (70 to 80 per cent) of propylene glycol in the droplets. Furthermore, the observed rate of evaporation of droplets of a propylene glycol mist is so rapid that a relatively high vapor concentration is liberated within a second or two.[6]

TABLE III

Effect of Propylene Glycol Vapor on Streptococcus hemolyticus

Vapor concentration	Time intervals of air samples	No. of colonies on plates from	
		Control chamber	Test chamber
1:7,700,000	Immediately after bacterial spray	370	36
	5 min. later	360	5
	15 min. later	312	0
	30 min. later	250	0
	60 min. later	96	0

(*c*) *Demonstration of the Bactericidal Activity of Propylene Glycol Vapor.*—Tests carried out under conditions identical with those in which the aerosol was employed, showed that propylene glycol vapor was not only highly bactericidal but acted more effectively than did the aerosol of this substance. In carrying out such experiments the chamber was filled with glycol vapor of known concentrations by means of one of the several methods described, following which the bacterial suspension was introduced. Concentrations of not less than 1 gm. of propylene glycol in three or four million cc. of air resulted in immediate and complete sterilization of the chamber air. This effect was demonstrated with staphylococci, pneumococci, hemolytic streptococci, *H. influenzae*, and *H. pertussis*. The results of an experiment in which pneumococcus Type I was employed as the test organism are exhibited in Plate

[6] The size and rate of evaporation of the droplets was determined by means of the Millikan oil-drop apparatus (18). These calculations together with a detailed discussion of the physicochemical interactions of propylene glycol gas molecules and fine fluid droplets will be presented elsewhere.

19, Figs. 1 to 6. With diminishing concentrations of propylene glycol, immediate and marked bactericidal activity was still obtained, although complete sterilization of the air required increasing intervals of time. Table III shows the lethal action of a vapor concentration of 1:7,700,000 (0.13 mg. per liter) on *Streptococcus hemolyticus*. The effectiveness of low concentrations of propylene glycol vapor was found to depend also on other variable factors, such as numbers of microorganisms dispersed into the air, the number of bacterial droplets, humidity of the atmosphere, state of bacterial suspension, etc. A detailed presentation of this phase of the work will be given in a second paper. Suffice it to say here that under optimum conditions pronounced bactericidal action of propylene glycol vapor against certain of the respiratory pathogens could be demonstrated in concentrations as small as 1 gm. of the glycol in 50,000,000 cc. of air.

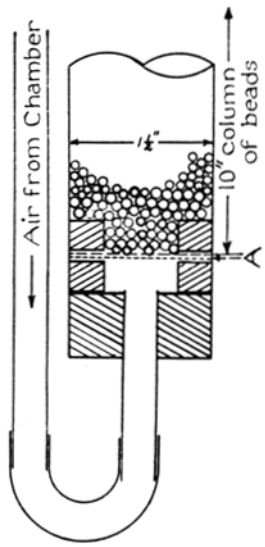
Propylene glycol vapor was also found to exert a lethal or at least an inactivating effect on the virus of influenza. This was determined by tests in which the presence of the glycol vapor in concentration of 1:3,000,000 was shown to protect mice completely against infection with amounts of air-borne influenza virus that produced death regularly in the control animals (19).

Tests with Other Glycols

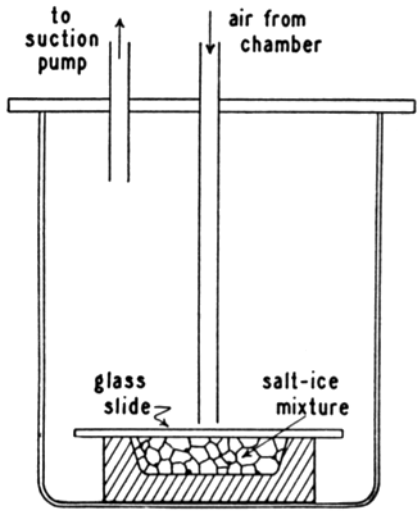
Experiments with other glycols in vapor form revealed variations in their bactericidal activity. Ethylene glycol, 2,3-butylene glycol, trimethylene glycol, and a number of compounds of related chemical composition were found to be highly effective. The relationship between chemical structure and bactericidal properties will be discussed in detail in a later communication dealing with the mechanism of the action of glycol vapors.

Evidence for the Bactericidal Action of Propylene Glycol

Other workers in this field have assumed that lack of growth on agar surfaces exposed to bacteria-laden atmospheres treated with germicidal mists represents actual death of the bacteria in the air. It seemed to us, however, that further experimental evidence was necessary to exclude the possibility that some factor or factors present in the air containing the germicidal mist or vapor might either inhibit growth or prevent adherence of the bacteria on the agar surface. In order to test for any growth-inhibiting effect due to condensation of the glycol itself on the collecting plates, the following control was performed: agar plates were exposed directly to air saturated with propylene glycol vapor or to the glycol spray from an atomizer, then used in taking samples from the control chamber. They yielded just as many colonies as plates not treated with glycol. The possibility that the reaction between the propylene glycol vapor and the bacterial droplets might somehow change the



TEXT-FIG. 3. Bead tower for collection of bacteria from chamber air. 20 to 25 cc. of broth diluted with equal parts of sterile water is placed in a 20 inch glass cylinder half filled with 3 mm. glass beads. This tower differs from that shown in Text-fig. 1 in that there are two monel metal screens of 24 and 70 mesh inserted at *A*.



TEXT-FIG. 4. Device for collecting air-borne bacteria on a glass slide.

state of suspension of the latter and prevent their adherence to the agar surface was tested by employing another method of collecting the vapor treated bacteria. Air from the chamber was drawn slowly through 25 to 50 cc. of diluted nutrient broth in a glass cylinder containing many small glass beads (Text-figs. 1 and 3). Plated samples of this fluid, through which air from the control chamber had been bubbled, yielded a large number of colonies, whereas samples of fluid through which the glycol-containing air had been drawn were sterile.

TABLE IV

Inoculation of Mice with Pneumococcus Type I Exposed to Propylene Glycol Vapor

	Material introduced into chamber	Air samples		No. of pneumococcus colonies		Mice inoculated with 1 cc. of fluid from bead tower
		Time taken	Method of culture	On plate	In fluid from bead tower	
Test chamber	Propylene glycol vapor. 1:3,000,000 followed by pneumococcus spray	Immediately after bacterial spray	Plate	0		
		10 min. later	Bead tower		0	10 mice. All remained well
		30 min. later	Plate	0		
Control chamber	Pneumococcus spray	Immediately after bacterial spray	Plate	1128		
		10 min. later	Bead tower		1 cc. = 228 Total = 5700*	10 mice. All died of pneumococcus infection in 24 to 36 hrs.
		30 min. later	Plate	484		

* The 2 liter air sample was drawn through 25 cc. of 50 per cent broth-water mixture.

The presence of killed bacteria in the glycol-treated air was demonstrated by condensing the moisture of the air drawn from the chamber on a chilled microscope slide, as shown in Text-fig. 4. When these preparations were stained, the bacteria appeared normal. Cultures of the condensed fluid on agar and in broth showed no growth.

We have also eliminated the possibility that microorganisms, although rendered incapable of growth on artificial media, might retain their capacity to reproduce in a suitable host. Experiments were conducted in which highly virulent pneumococci Type I were sprayed into a chamber containing the propylene glycol vapor. The air was then drawn through sterile broth in a bead tower and 1 cc. quantities of this fluid were injected into mice. These

animals survived. However, when the procedure was performed with air drawn from the control chamber, all the mice died of pneumococcic infection. The protocol of an experiment of this type is shown in Table IV.

Further evidence of the bactericidal action of propylene glycol vapor was provided by cultures made from the floor and walls of the chamber at the end of the experiment. Whereas cultures from the control chambers always yielded large numbers of bacteria, those from the test or glycol-treated chamber were uniformly sterile.

Additional Data and Comments

The question as to how propylene glycol produces its rapid and marked bactericidal effect has not been elucidated. However, certain observed characteristics of the glycol-treated microorganisms indicate something of the general nature of the effect. Gram-positive bacteria killed by exposure to propylene glycol, either in vapor or liquid form, retain their Gram-positiveness as well as their morphological integrity. Glycol-killed pneumococci show typical capsular swelling in the presence of specific antipneumococci rabbit serum and retain their antigenic properties. Mice, vaccinated with pneumococci killed by propylene glycol, were found to be just as resistant to the injection of living microorganisms as were mice similarly immunized with heat-killed pneumococci. While pneumococci suspended in propylene glycol retain their Gram-positiveness for many weeks or months, removal from this medium results in their becoming Gram-negative. Such microorganisms freed from the glycol undergo gradual dissolution. Autolysis is hastened by the presence of bile salts. The change from the Gram-positive to the Gram-negative state can be brought about by simply adding an equal volume of water or physiological salt solution to the glycol suspension. These findings indicate that propylene glycol inhibits but does not destroy the autolytic enzyme system of the pneumococcus cell. Whether or not the other enzyme systems of the pneumococcus are affected remains to be determined.

We have not made comparative studies of the effectiveness of propylene glycol and the several compounds and mixtures previously employed by other workers, except in the case of Twort's "S^2". Our observations with "S^2" (10 per cent hexylresorcinol in alkaline propylene glycol) have been confined to tests on *Staphylococcus albus*. These experiments showed that the addition of hexylresorcinol to propylene glycol increased markedly the bactericidal activity of the latter substance. The difference between the two agents both in aerosol and vapor forms was slight as judged by the immediate sterilizing effect, but became apparent after 5 to 15 minutes. An increased lethal action might have been anticipated from the presence of hexylresorcinol since, *in vitro*, this substance has been shown to be bactericidal in dilutions of between 1:2,000 and 1:20,000 (depending on the manner of testing) in contrast to

propylene glycol which requires a concentration of 1:1, *i.e.*, equal parts of bacterial suspension and glycol to produce a similar effect. Even higher concentrations of propylene glycol were needed to kill certain non-pathogenic microorganisms. Since rapidity of action of the air sterilizing agent would seem to be of great importance in the application of such a method to the control of air-borne droplet infection, and since the addition of hexylresorcinol appears to contribute little to the immediate bactericidal effect of propylene glycol, we feel that for this and other reasons the use of the latter substance alone is to be preferred. However, it may be desirable to employ, for certain purposes, highly bactericidal agents such as hexylresorcinol even though they possess much greater potential toxicity than does propylene glycol.

Atmospheres containing propylene glycol vapor in concentrations up to the saturation point (0.7 mg. of glycol per liter of air or 1:1,400,000) are invisible, odorless, non-irritating, and tasteless, except in the highest concentrations when a faintly sweetish taste is detectable. Tests on the toxicity of propylene glycol administered by the usual routes, *i.e.*, oral and intravenous, have shown this substance to be essentially non-toxic (20). While it would seem probable that propylene glycol taken into the body by way of the respiratory tract would be equally innocuous, such an assumption is not justifiable in the absence of direct experimental evidence. We have carried out tests on the effect of maintaining rats in atmospheres of propylene glycol vapor for a year or more. The results of these experiments will be presented in the second paper.

SUMMARY

It has been found that propylene glycol vapor dispersed into the air of an enclosed space produces a marked and rapid bactericidal effect on microorganisms introduced into such an atmosphere in droplet form. Concentrations of 1 gm. of propylene glycol vapor in two to four million cc. of air produced immediate and complete sterilization of air into which pneumococci, streptococci, staphylococci, *H. influenzae*, and other microorganisms as well as influenza virus had been sprayed. With lesser concentrations of propylene glycol, rapid and marked reduction in the number of air-borne bacteria occurred, but complete sterilization of the air required a certain interval of time. Pronounced effects on both pneumococci and hemolytic streptococci were observed when concentrations as low as 1 gm. of glycol to fifty million cc. of air were employed.

Numerous control tests showed that failure of the glycol-treated microorganisms to grow on the agar plates was due to actual death of the bacteria. The means by which propylene glycol vapor produces its effect on droplet-borne bacteria is discussed and data relating the bactericidal properties of propylene glycol *in vitro* to the lethal action of its vapor is presented.

Atmospheres containing propylene glycol vapor are invisible, odorless,

and non-irritating. This glycol is essentially non-toxic when given orally and intravenously. Tests on possible deleterious effects of breathing propylene glycol containing atmospheres over long periods of time are being carried out.

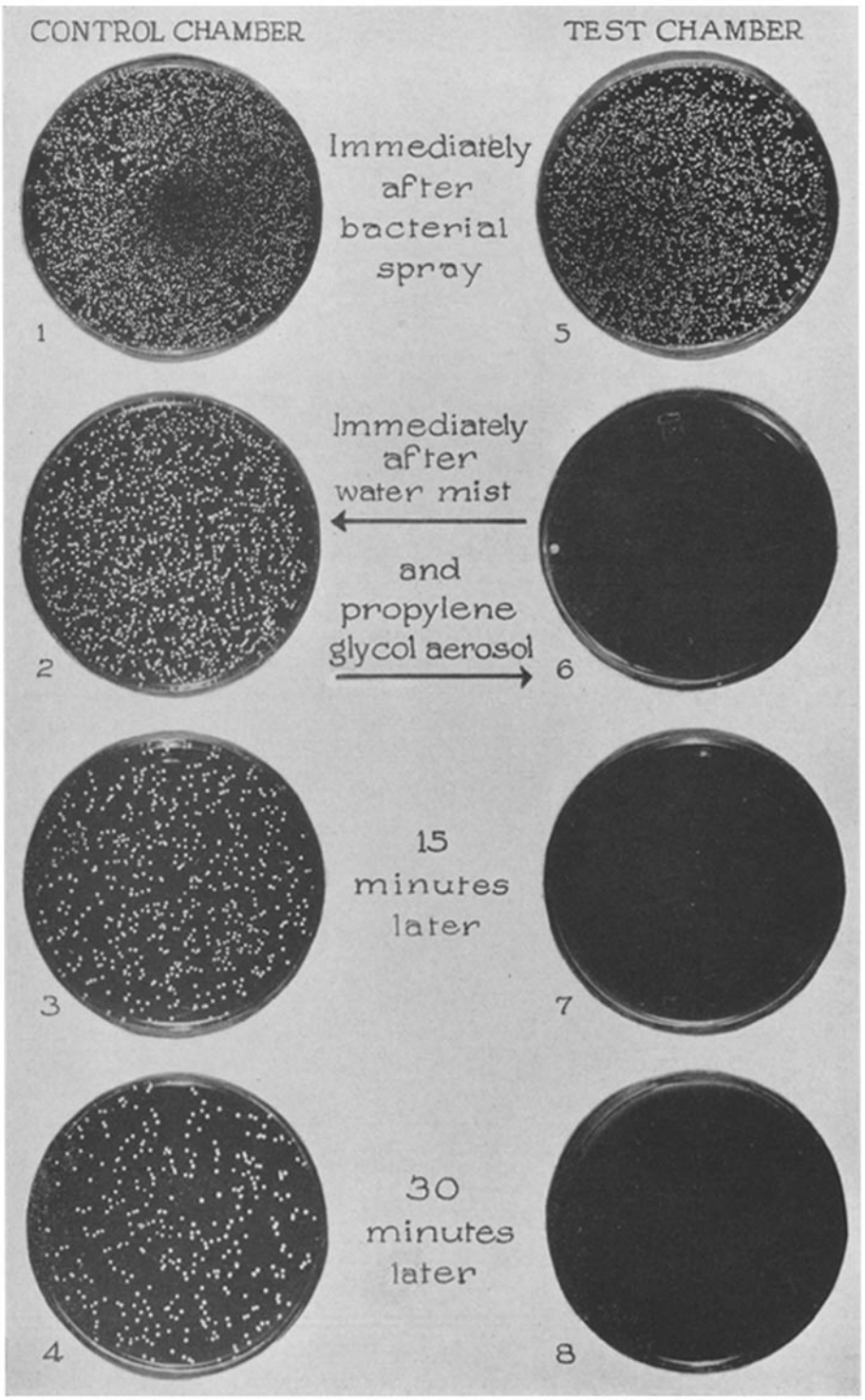
BIBLIOGRAPHY

1. Douglas, S. R., Hill, L., and Smith, W., *J. Ind. Hyg.*, 1928, **10,** 219.
2. Trillat, M. A., *Bull. Acad. méd.*, 1938, series 3, **119,** 64.
3. Masterman, A. T., *J. Ind. Hyg.*, 1938, **20,** 278.
4. Masterman, A. T., *J. Hyg.*, Cambridge, Eng., 1941, **41,** 1, 44.
5. Pulvertaft, R. J. V., and Walker, J. W., *J. Hyg.*, Cambridge, Eng., 1939, **39,** 696.
6. Twort, C. C., Baker, A. H., Finn, S. R., and Powell, E. O., *J. Hyg.*, Cambridge, Eng., 1940, **40,** 253.
7. Andrewes, C. H., *Lancet*, 1940, **2,** 770.
8. Twort, C. C., and Baker, A. H., *Lancet*, 1940, **2,** 587.
9. Robertson, O. H., Bigg, E., Miller, B. F., and Baker, Z., *Science*, 1941, **93,** 213.
10. Miller, B. F., and Baker, Z., *Science*, 1940, **91,** 624.
11. Robertson, O. H., Bigg, E., Miller, B. F., Baker, Z., Puck, T. T., *Tr. Assn. Am. Physn.*, 1941.
12. Robertson, O. H., Bigg, E., Miller, B. F., Puck, T. T., and Baker, Z., in Moulton, F. R., Aerobiology symposium, The American Association for the Advancement of Science, 1942, No. 17, in press.
13. Baker, A. H., and Twort, C. C., *J. Hyg.*, Cambridge, Eng., 1941, **41,** 117.
14. Graeser, J. B., and Rowe, A. H., *Am. J. Dis. Child.*, 1936, **52,** 92.
15. Hollaender, A., and DallaValle, J. M., *Pub. Health Rep., U. S. P. H. S.*, 1939, **54,** 1, 574.
16. Lehman, A. J., and Newman, H. W., *J. Pharmacol. and Exp. Therap.*, 1937, **60,** 312.
17. Puck, T. T., *Science*, 1942, **95,** 178.
18. Millikan, R. A., The electron, Chicago, The University of Chicago Press, 1924, 90.
19. Robertson, O. H., Loosli, C. G., Puck, T. T., Bigg, E., and Miller, B. F., *Science*, 1941, **94,** 612.
20. Hanzlik, P. S., Newman, H. W., Van Winlke, W., Lehman, A. J., and Kennedy N. K., *J. Pharmacol. and Exp. Therap.*, 1939, **67,** 101.

EXPLANATION OF PLATES

PLATE 18

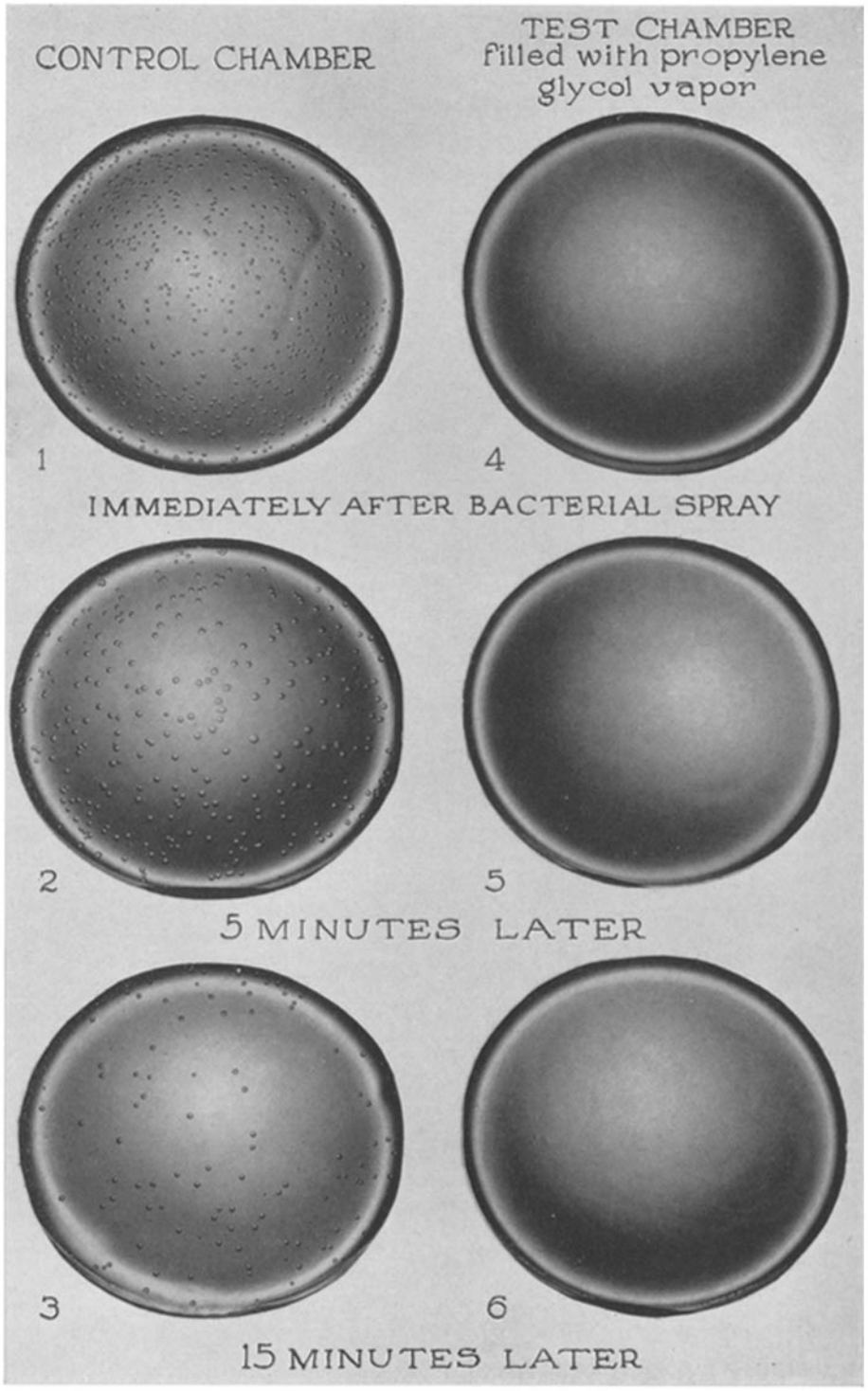
FIGS. 1 to 8. The effect of propylene glycol aerosol on *Staphylococcus albus*. The same quantity of a 1:10 dilution of the standard bacterial suspension was sprayed into each chamber. Figs. 1 to 4 are photographs of plate samples taken from control chamber. Figs. 5 to 8 show the results of air samples taken before and after the introduction of propylene glycol aerosol in a concentration of 1 gm. of glycol in 2,000,000 cc. of air. Plate 6 showed only one colony. 7 and 8 were sterile. The air samples represented by plates 2 and 6 were begun within 2 or 3 seconds after the termination of the water and glycol mists respectively.



(Robertson *et al.*: Bactericidal action of propylene glycol vapor. I)

PLATE 19

FIGS. 1 to 6. The effect of propylene glycol vapor on pneumococcus Type I. Equivalent amounts of a 1:20 dilution of the standard bacterial suspension were sprayed into each chamber. Figs. 1 to 3 show plates exposed to air samples from the control chamber. Plates exposed to air samples from the test chamber which was filled beforehand with air containing propylene glycol vapor in a concentration of approximately 1 gm. of glycol in 3,000,000 cc. of air, were uniformly sterile, Figs. 4 to 6.



(Robertson *et al.*: Bactericidal action of propylene glycol vapor. I)