

THE MECHANISM OF AERIAL DISINFECTION BY GLYCOLS AND OTHER CHEMICAL AGENTS

II. AN ANALYSIS OF THE FACTORS GOVERNING THE EFFICIENCY OF CHEMICAL DISINFECTION OF THE AIR*

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(Received for publication, January 31, 1947)

In the preceding paper of this series (1) it was shown that rapid killing of air-borne microorganisms requires that the chemical agent be dispersed as a vapor in the atmosphere. It follows, then, that the effectiveness of any substance as an aerial germicide depends directly on the degree of condensation of its vapor on the air-suspended particles, and on the rate at which the resulting concentration of germicide can bring about death of the microorganisms. The present paper deals largely with an attempt to formulate the theoretical relationships underlying this process. Some new experimental data will be presented and observations already reported in the literature will be correlated in terms of the vapor condensation concept.

In general, there would seem to be only three processes by which condensation can occur on air-borne droplets: solution of the vapor within the particle, adsorption of the vapor upon the surface of the particle, or the precipitation of a supersaturated vapor upon particles wherein neither of these processes occurs to any appreciable extent. The present discussion will be limited almost exclusively to the first of these mechanisms, so that we shall be concerned mainly with bacteria suspended in moist droplets, in which aerial disinfectants like the glycols, are highly soluble. The necessary thermodynamic condition which must be fulfilled in order for a soluble vapor to condense in a droplet is that the pressure of the vapor in the air must exceed its partial pressure inside the droplet, i.e.

$$p_g > p_g^0 \gamma_g (1 - \Sigma N_i) \quad (1)$$

where p_g = the pressure of the germicide vapor present in the atmosphere; p_g^0 is the vapor pressure of the pure germicide at the prevailing temperature; γ_g is the activity coefficient of the germicide, and ΣN_i represents the summation of the mole fraction of all other soluble components in the droplet.

* This work has received support from the Commission on Air-Borne Infections, Army Epidemiological Board, Preventive Medicine Service, Office of the Surgeon General, United States Army; the United States Public Health Service; and the Bartlett Memorial Fund of the University of Chicago.

1. Effect of the Vapor Pressure of the Disinfectant

Equation 1, defining the necessary condition without which condensation is impossible, indicates the rôle played by p_a^0 , the vapor pressure of the pure germicide. The higher this vapor pressure, the greater is the concentration required for effective bactericidal action in the air. This arises from the tendency of a vapor to distribute itself between a gas phase and a solution so that its relative saturation¹ is the same in the two phases. For highly volatile substances, therefore, it is necessary to maintain a great deal more material in the air in order to achieve the same degree of saturation, and hence, the same extent of condensation on suspended droplets, as can be obtained with a small concentration of a less volatile material.

To demonstrate this influence of the vapor pressure on the critical atmospheric concentration necessary for effective bactericidal action, a series of experiments was performed, comparing the potency of four compounds, ethyl alcohol, propylene glycol, dipropylene glycol, and triethylene glycol, in killing air-borne microorganisms.

Tests were carried out in the tightly sealed, glass-walled experimental room 640 cubic feet in capacity (2) which was maintained at a temperature of 72°F. and a relative humidity of 28 per cent throughout the entire series. Vaporization of measured quantities of each compound was effected from a Petri dish resting on a small hot plate which was operated by a switch outside the chamber. A fan maintained a slow, uniform movement of the chamber air throughout each experiment. As soon as vaporization was complete, a measured amount of a suspension of *Staphylococcus albus* in broth was sprayed into the chamber according to the standard technique already described (2). Settling plates were exposed for successive 5 minute intervals, starting with the beginning of the bacterial spray and continuing for 30 minutes.² Every experiment was followed by a control, and the numbers of bacteria recovered on the test plates were compared to those obtained during the corresponding periods of the control run, so that per cent reductions with respect to the control could be calculated. Many of these experiments were run in duplicate and the agreement so obtained was highly satisfactory.

For each substance, the quantity of material vaporized was progressively increased in successive experiments until the minimum amount which would produce death of 95 per cent of the air-borne microorganisms within 10 minutes, was established. These data are presented in Table I. They show an unmistakable increase in the minimum killing concentration as the vapor pressure of the substance employed increases.

All of the compounds listed in Table I are miscible with H₂O and possess similar chemical groupings. Their bactericidal properties *in vitro* as judged by the concentrations needed to kill standard suspensions of microorganisms, are

¹ Strictly speaking, it is the escaping tendency which, at equilibrium, is the same in the two phases.

² We have previously demonstrated that exposure of settling plates in a uniform and constant current of air such as obtains in these chambers, is an excellent air-sampling method producing results parallel to that of the bubbler samples, but even more uniform (2).

very nearly alike within several per cent (3). Yet the concentration required for effective air disinfection varies many thousandfold and follows the same order as their vapor pressures.

From a practical standpoint, there is an obvious advantage attached to the use of compounds of low vapor pressure for aerial disinfection since much smaller quantities will be required to produce a desired killing action. Another important practical feature lies in the consideration of the fact that condensation of the disinfectant vapors

TABLE I
Effect of Vapor Pressure of the Disinfectant (T = 72°F. Relative Humidity=28 Per Cent)

Substance	Vapor pressure at 72°F.		Minimum vapor concentration necessary to kill 95 per cent of air-borne <i>Staphylococcus albus</i> within 10 min.	
			Amount vaporized in 640 ft. ³ chamber	Theoretical concentrations in the air*
	<i>mm. Hg.</i>	<i>mg./liter</i>	<i>gm.</i>	<i>mg./liter</i>
Triethylene glycol.....	0.001 (4)	0.0087	0.11	0.0060
Dipropylene glycol.....	0.02 (32)	0.15	0.49	0.027
Propylene glycol.....	0.1 (4)	0.41	3.4	0.19
Ethyl alcohol.....	49.2 (33)	123.0	>300‡	>16

* The figures presented here are valid for comparative purposes only, because this theoretical concentration is calculated on the assumption that all the material vaporized is available for bactericidal action. Actually, the effective concentration of disinfectant which obtains in the air under these conditions is less than this amount, because some vapor is being continuously removed from the air by condensation on air-borne particles, by adsorption on the chamber walls, and by diffusion out of the chamber through small leaks.

‡ This was the highest value tested because it was feared that the explosive limit might be reached. Experiments performed in the small chambers demonstrated that saturated atmospheres of ethyl alcohol kill 95 per cent of air-borne staphylococci in less than 1 minute.

may occur on any cold surfaces inside of rooms whose air is to be treated. Use of compounds with low vapor pressures insures that such condensation will be small, whereas extensive precipitation would occur with more volatile compounds.

The vapor pressure of a useful aerial disinfectant must not be too low, however, or else the rate of kill will be seriously reduced. The velocity of condensation of vapor molecules on air-suspended droplets depends upon the rate of diffusion of these molecules into the vicinity of the droplet, and on the rate of collision between the droplet and the layer of vapor molecules surrounding it ((1), Appendix, Equation 6). Both of these processes depend directly on the number of vapor molecules present per cubic centimeter of the atmosphere. The lower the vapor pressure of the compound employed, the smaller will be the maximum molecular concentration which can be maintained in the air.

Therefore, the rate of condensation will be decreased, the attainment of the equilibrium concentration within the droplets will occur more slowly, and the killing action will be delayed. Thus, one may expect that although triethylene glycol is effective in lower concentrations than propylene glycol, its rate of action will be slower than that of propylene glycol at the same percentage saturation in the air.³

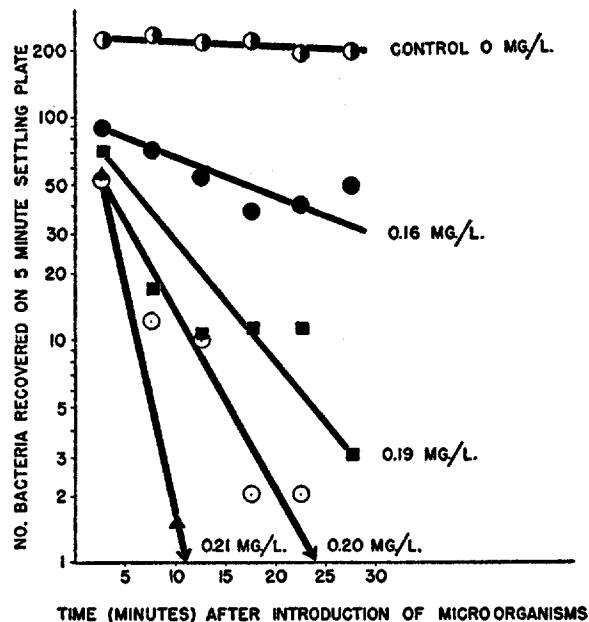


FIG. 1. Logarithmic plot showing survival of a suspension of *Staphylococcus albus* sprayed into a chamber containing various concentrations of propylene glycol vapor.

2. Effect of Varying the Concentration of Germicide Vapor in the Air

When a germicide vapor is present in the air in an amount sufficient to initiate condensation, its concentration in this condensate should depend directly upon its concentration in the air. This follows from the laws of solution, *i.e.*

$$N_g = \frac{p_g}{p_g^o \gamma_g} \quad (2)$$

where N_g is the equilibrium concentration of germicide in the liquid expressed as the mole fraction; p_g is the partial pressure of germicide in the air; p_g^o is its

³ The importance of having accurate vapor pressure data for these compounds has made necessary the development of methods for measurement of vapor pressures of compounds of very low volatility. Quantitative studies of the vapor phase equilibria of one and two component systems involving such compounds have been carried out (4).

vapor pressure, and γ_s is its activity coefficient in the solution so formed. An increased concentration of germicide in the condensate should be reflected in a higher rate of bactericidal action. Quantitative experimental observations were in agreement with this predicted behavior. In the experiments described in the preceding section, it was found that for each compound tested, the rate of bactericidal action increased when higher concentrations of germicide vapor were employed. In Fig. 1 are shown a set of representative data for propylene glycol. Triethylene and dipropylene glycols exhibited similar behavior. The killing action of each of these compounds under the prescribed experimental conditions could be represented within the range of experimental error by the following equation:

$$\text{Log } N = \log N_0 - kc^4t \quad (3)$$

where N = number of microorganisms per liter of air present at any time t , after the end of the spray, N_0 = number of microorganisms per liter of air at $t = 0$, k = a constant, specific for each disinfectant, c = calculated concentration of disinfectant vaporized into the room in milligrams per liter. t = time in minutes.

The values of k so obtained are as follows: triethylene glycol 4.5×10^7 ; dipropylene glycol 2.4×10^6 ; and propylene glycol 65.3.

3. Effect of Hygroscopicity of the Germicide

The ubiquitous presence of water vapor in the air makes possible a much more effective killing action, if the germicide employed be hygroscopic. This increased efficiency occurs because the existence of a high affinity between water molecules and those of the lethal agent results in their mutual condensation at concentrations below that at which either one would precipitate, if present alone. This action may be made clear by the following analysis. Consider an atmosphere of a given water vapor level, containing moist droplets in suspension. The moisture content of these droplets, at equilibrium, is determined by the relative humidity, for droplets freshly sprayed into the atmosphere will either lose water by evaporation, or gain it by condensation until the partial pressure of water inside the droplet is equal to the aqueous tension of the air.⁴ If the vapor of triethylene glycol, for example, is now admitted into such a system, glycol molecules will dissolve in the aqueous droplet, because of the high affinity between these molecules and water. But this addition of glycol to the droplet results in a lowering of the partial pressure of water in it, so that the droplet is no longer in equilibrium with the atmospheric humidity. Hence, more water vapor from the air must again condense on the droplet, in order to restore the water balance which was upset by the introduction of the glycol.

⁴ In a very dry atmosphere, and in the absence of highly soluble material dissolved in the droplets they may evaporate to dryness.

In other words, the introduction of glycol vapor into an atmosphere containing suspended droplets, results in the simultaneous condensation of both water and glycol vapors on to the droplets, ultimately producing a composition which is in equilibrium with both the glycol vapor and the water vapor in the atmosphere. These relationships are illustrated in Fig. 2. The more hygroscopic the germicide, the more extensively will its vapor condense in moist droplets, and the smaller will be the partial pressure required for efficient aerial disinfection.

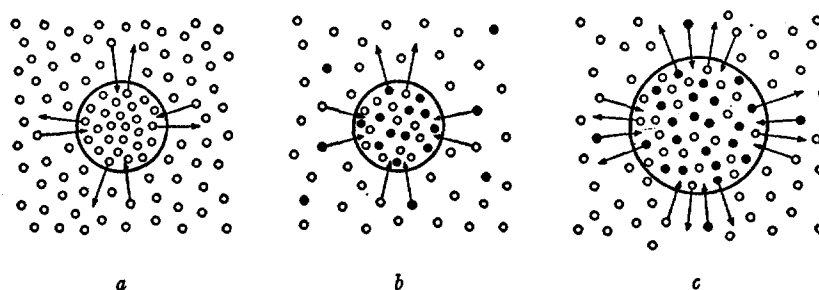


FIG. 2. (a) Droplet in equilibrium with H_2O vapor of the air. Number of water molecules escaping equals number of water molecules condensing.

(b) Glycol vapor added condenses on the droplet, and impedes the evaporation of water. Hence, more water molecules from the atmosphere condense than are reevaporated so the water content of the droplet also increases.

(c) Final equilibrium condition is reached when both water and glycol have been added to the droplet until the concentration of each of these components in the liquid phase is in equilibrium with their respective vapors. Note that the final size of the droplet is now increased because of the addition of two components.

On the other hand, if a completely insoluble vapor is employed condensation will not occur for any partial pressure less than the pure vapor pressure of the compound, unless specific adsorptive forces exist between the germicide and some component of the bacterial droplet other than water. Whether or not condensation of a water-insoluble germicide will ever occur on moist bacterial particles will depend upon the degree of supersaturation which can be achieved in the atmosphere, and on the intensity of any adsorptive forces which may be operative.

The rôle of hygroscopicity in decreasing the concentration of germicide vapor required for effective bactericidal action can be illustrated by a comparison of the behavior of some representative compounds. Chlorine and $HOCl$ are both easily vaporizable substances, possessing high intrinsic toxicity for bacterial metabolism. They differ markedly in their affinity for water, however, the latter possessing a high solubility whereas that of the former is slight. A corresponding difference exists in the effectiveness of their vapors as aerial dis-

infectants, for whereas free Cl_2 exhibits little or no bactericidal action even in fairly high concentrations, HOCl vapor is highly lethal even when very dilute (5-7).

A similar correlation is evident in the behavior of organic hydroxy compounds. Propylene glycol and triethylene glycol are miscible with water in all proportions. Both are excellent aerial bactericides, producing complete and rapid killing of air-borne microorganisms even when present in concentrations far below their saturation pressures (8, 9). Their behavior in this respect is to be contrasted with that of lauryl alcohol, which possesses a saturated vapor density intermediate between those of the two former compounds,⁵ and which is much more potent than either of these as an inhibitor of bacterial metabolism *in vitro* (3). Yet lauryl alcohol is a very inefficient aerial germicide. Experiments were carried out in which lauryl alcohol was vaporized into the experimental chambers in quantities equivalent to supersaturated concentrations. Under these conditions, the best bactericidal action which could be attained was a killing of 60 per cent of a standard dispersion of air-borne hemolytic streptococci in 20 minutes.

4. Effect of the Relative Humidity of the Atmosphere

Various and sometimes conflicting rôles have been ascribed to the action of the atmospheric relative humidity in promoting or retarding the killing action of aerial disinfectants (10-13, 6). The vapor condensation concept permits an explanation of the influence of the relative humidity by means of an analysis of the equilibrium which is established between the liquid and vapor phases of the system (8, 14).

The combination of a soluble bactericidal agent and water, simultaneously present in both the vapor state and in liquid droplets, constitutes a system whose composition can be predicted by means of a phase diagram. For the sake of clarity, we shall divide this discussion into two parts first considering that no other soluble materials are present in the air-suspended droplets other than water and the germicidal agent. Then the effects of the presence of foreign substances, dissolved in bacterial droplets, will be determined.

In Fig. 3 are plotted the partial pressures of H_2O vapor (line B) and any water-miscible germicide vapor (line A) respectively which are in equilibrium with aqueous solutions containing various concentrations of germicide.

These equilibrium relationships may be expressed as follows:

$$\begin{aligned} p_g &= p_g^0 N_g \gamma_g \\ &= p_g^0 \gamma_g (1 - N_{\text{H}_2\text{O}}) \end{aligned} \quad (4)$$

⁵ Thanks are due to Dr. Elizabeth S. Horning, of Dupont de Nemours and Company, for supplying the sample of lauryl alcohol, and to Mr. Edward Dunklin of this laboratory, who found the value for its saturated vapor density at 25°C. to be 10.8×10^{-3} mg. per liter.

since the solution is also in equilibrium with respect to water.

$$\begin{aligned} \text{But, } N_{\text{H}_2\text{O}} &= \frac{p_{\text{H}_2\text{O}}}{p_{\text{H}_2\text{O}}^{\circ} \gamma_{\text{H}_2\text{O}}} \quad \text{from the laws of solution} \\ &= \frac{RH}{\gamma_{\text{H}_2\text{O}}} \quad \text{where } RH \text{ is the relative humidity.} \end{aligned} \quad (5)$$

Hence,

$$\begin{aligned} p_g &= p_g^{\circ} \gamma_g \left(1 - \frac{RH}{\gamma_{\text{H}_2\text{O}}} \right) \\ &= p_g^{\circ} \quad (\text{maximum}) \end{aligned} \quad (6)$$

This equation (6) defines the maximum amount of any water-soluble germicidal agent which can exist in the vapor state, at any relative humidity.

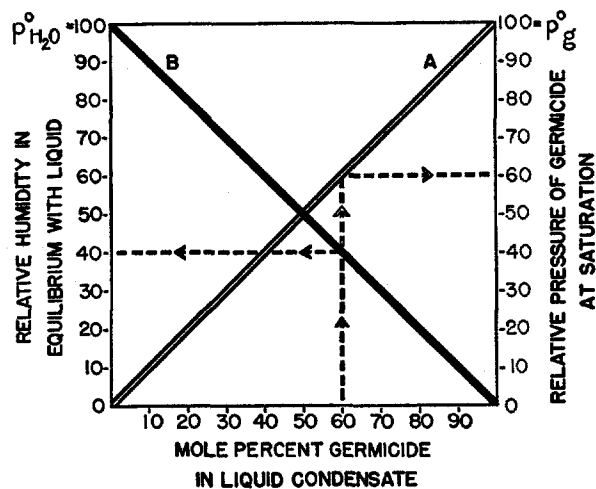


FIG. 3. Liquid-vapor composition diagram for a two phase, two component system, where water is one component, and any aerial bactericidal agent is the other. The bactericidal agent, G , must be completely miscible with H_2O , and it is assumed that both components obey Raoult's law within tolerable limits. Thus, this diagram could refer equally well to propylene glycol, triethylene glycol, lactic acid, etc.

$p_{\text{H}_2\text{O}}^{\circ}$ = vapor pressure of pure H_2O

p_g° = vapor pressure of the pure bactericidal agent.

The line A gives the maximum partial pressure of bactericide vapor in equilibrium with any liquid solution whose composition is given by the abscissa. Similarly the line B gives the partial pressure of water vapor which is in equilibrium with this same solution.

From a consideration of the relationships shown in Fig. 3, the following facts emerge:

(a) *The Saturation Concentration of Germicide Vapor Decreases as the Rela-*

live Humidity Increases.—The lines A and B of Fig. 3 define the maximum concentration of germicide vapor and water vapor respectively which can coexist in the atmosphere. The presence of water vapor decreases the ability of the air to support other water-soluble vapors. Only at zero relative humidity can the air contain an amount of germicide vapor equivalent to the vapor pressure of the pure compound. Similarly at 100 per cent relative humidity, no germicide whatever can exist in the vapor state.

In such a two component system, condensation will occur only when the saturation concentrations indicated in the diagram are exceeded. Thus, at a relative humidity of 40 per cent, condensation of germicide will not occur unless it is present at a pressure greater than 60 per cent of its pure vapor pressure and the initial composition of the liquid condensate under these conditions would consist of 60 mole per cent of germicide.

(b) *Under Conditions of Practical Application the Relative Humidity Controls the Maximum Concentration of Germicide Which the Air Can Contain but the Converse Relationship Does Not Hold.*—Thus an increase in relative humidity of an atmosphere already saturated with germicide vapor, causes condensation to occur with a consequent decrease in the concentration of the germicide vapor, until a new level is reached compatible with the new relative humidity. But it is not feasible to decrease the relative humidity of the air appreciably, by continued introduction of germicide vapor, because of the great disparity in amount of these two substances required for saturation. Thus, in an atmosphere of 50 per cent relative humidity, which is also saturated with triethylene glycol vapor, there exist about 20,000 times as many water molecules as there are triethylene glycol molecules. The initial composition of the condensate which would occur if the concentration of either component in such an atmosphere is increased, would be an equimolecular solution of glycol in water (Fig. 3). Hence, it would be necessary to introduce into the air quantities of glycol vapor thousands of times greater than its saturation value, in order for these molecules to combine with and remove from the vapor phase enough water molecules to appreciably lower the relative humidity. Such a procedure would cause intense fogging of the air, and would waste vast quantities of the germicidal agent.

(c) *By Controlling the Maximum Concentration of Germicidal Vapor Which Can Exist in the Air, the Relative Humidity Also Determines the Maximum Concentration of Germicide Which Can Accumulate within the Droplets.*—Equation (2) shows that the mole fraction of germicide in liquid aerosol droplets is equal to the ratio of the partial pressure of the germicide which exists in the air, to its pure vapor pressure.⁶ By limiting the maximum value of the partial pressure which can exist in the air, the relative humidity also limits the maximum concentration of germicide which can exist in solution in the droplets. At high relative humidities, very little vapor can be maintained in the atmosphere.

⁶ (Assuming unit activity coefficients.)

Hence, the maximum value of p_g is small and the concentration of germicide which can accumulate in the droplets is also small. Experimental data showing that the bactericidal action of both propylene and triethylene glycols is markedly diminished at relative humidities greater than 60 per cent have already been presented (8, 12).

5. Effect of Presence of Dissolved Substances in the Bacterial Droplets

The preceding discussion applied only to the effect of relative humidity in influencing condensation of molecules of germicide and molecules of pure water to form droplets containing no other dissolved material. We shall now consider the behavior of air-borne bacterial particles which are associated with at least small amounts of other soluble substances. This would seem to be by far the most common state of naturally occurring bacterial particles.

As before, germicide vapor will condense when its concentration in the air exceeds that partial pressure which is in equilibrium with these particles. Thus, we can now write equation (1) the condition for condensation to occur as:

$$p_g > p_g^o \gamma_g (1 - N_{H_2O} - N_x) \quad (7)$$

and by combination with (5)

$$p_g > p_g^o \gamma_g \left(1 - \frac{RH}{\gamma_{H_2O}} - N_x \right) \quad (8)$$

where N_x represents the combined mole fractions of all the other components of the solution. It is apparent from comparison of this equation with Equation 6 that now *glycol vapor can condense on air-borne particles even when its concentration in the air is less than that required to saturate the atmosphere at the prevailing relative humidity.*

The magnitude of the effect of these dissolved substances can be appreciated when the dynamics of the origin of these bacterial particles is considered. Bacteria expelled from human respiratory tracts are introduced into the atmosphere suspended in a solution containing small amounts of dissolved salts, proteins, and other components. These droplets can lose their moisture with tremendous rapidity, so that the various solutes present may become highly concentrated. If the relative humidity is sufficiently low, they may even evaporate to dryness. Thus, although the total amount of dissolved material may be small, its mole fraction in the droplet (N_x) may be quite high. If the germicidal vapor is soluble in the material composing the bacterial particle, it will condense, regardless of how dilute its original concentration in the air.

We can calculate the concentration of germicide which will accumulate in such droplets, at any relative humidity, and for any partial pressure of the germicide:

Let S = degree of saturation of germicide vapor in the atmosphere, defined, as the ratio of its concentration in the air to the maximum concentration which can exist at the prevailing relative humidity.

Then

$$S = \frac{p_g}{p_g \text{ (maximum)}}$$

and from (6)

$$S = \frac{p_g}{p_g^o \gamma_g \left(1 - \frac{RH}{\gamma_{H_2O}}\right)} \quad (9)$$

Therefore,

$$\frac{p_g}{p_g^o} = S \gamma_g \left(1 - \frac{RH}{\gamma_{H_2O}}\right) \quad (10)$$

But also

$$\frac{p_g}{p_g^o} = \gamma_g N_g \quad (11)$$

So that

$$N_g = S \left(1 - \frac{RH}{\gamma_{H_2O}}\right) \quad (12)$$

Equation (8) indicates the necessary condition in order for condensation to occur. When this condition is fulfilled, equation (12) represents the cumulative effect of relative humidity and concentration of germicidal vapor in determining the concentration of the lethal agent in this liquid condensate.

If the bacterial droplets contain no substance miscible with water, they can become completely desiccated if the relative humidity falls to a sufficiently low value. Under these conditions, the high water affinity of the germicidal vapor, which causes it to condense on moist particles even when its concentration in the air is extremely low, becomes ineffective; it may even resist condensation on such dried particles when present at the saturation level. This will occur when the surface which the particle presents to the atmosphere offers but little attraction for the molecules of the germicide (15). Experimental measurements bearing out this interpretation have been described in an earlier publication (8).⁷

Throughout this discussion of the effects of humidity it has been implicit that each bacterial droplet actually achieves equilibrium with the atmosphere very rapidly, so that its composition is equal to that given by the equation (12). For small, moist particles this assumption is justified in practice since studies have shown that the rate of interaction of such droplets with the sur-

⁷ It is impossible to set a limiting value for the relative humidity at which desiccation occurs, because of its dependence on the chemical composition of the dissolved substances in the droplets, which may vary considerably in different situations. In general, a droplet will dry out completely when the relative humidity is less than the aqueous tension of a saturated solution of its most soluble component.

rounding atmosphere is extremely rapid (16). The high hygroscopicity of compounds like those of propylene or triethylene glycols further insures the rapidity of the process because of the great affinity of such molecules for water. Only the existence of large potential barriers could significantly decrease the collision efficiency of such molecules with aqueous droplets. The fact that these vapors produce extensive bactericidal action within 15 seconds or less is in itself sufficient indication that the velocity of the condensation process is rapid enough to enable equilibrium conditions to be reached.

6. Influence of the *in Vitro* Germicidal Potency of the Disinfectant

It was early recognized by workers investigating aerial disinfection, that the killing efficiency of a series of compounds in the air does not follow the same order as their phenol coefficients in the test tube (17, 5). However, the reason for this apparent anomaly never received a satisfactory explanation. In the present discussion, we have shown that a number of factors operate to control the concentration of disinfectant which is deposited upon air-borne bacterial particles. In the light of this analysis, the rôle played by the phenol coefficient of the compound employed, may readily be understood.

The rate of action of most germicides varies directly with the concentration to which the microorganisms are exposed. It is obvious, therefore, that the lower the intrinsic toxicity of any compound for bacterial metabolism, the greater must be its concentration in the condensate which forms on air-suspended droplets if it is to produce rapid lethal action. Hence, whereas a highly bactericidal compound like *n*-hexyl resorcinol (17) can produce rapid killing when present in great dilution in a bacterial droplet, a substance with little or no toxicity for bacterial metabolism may produce no lethal action, even if it accumulates in concentrated solution about the microorganisms.

The influence of variations in the phenol coefficients of different compounds, can only be compared when all the factors which govern the extent of condensation are also controlled so that in each case, the microorganisms are exposed to approximately the same concentration of germicide. This has been done for the compounds listed in Table II where the vapor pressures, solubility in water (which in the absence of more specific data, will be used as an index of the water affinity of these compounds), and the time necessary for a fixed concentration of each molecular species to kill streptococci *in vitro*, are presented. The physical properties of the first three compounds in this table resemble each other fairly closely. They are all highly hygroscopic, and their volatilities are of the same order of magnitude. Hence, when vaporized under the same conditions of temperature and humidity, they should be expected to condense to approximately the same extent upon moist bacterial droplets. A series of experiments testing their germicidal action in the air, revealed that the efficiencies of these compounds as aerial disinfectants reflect the differences in their bactericidal

action *in vitro*. Thus, whereas triethylene glycol vapor even in concentrations far below the saturation point kills air-borne beta hemolytic streptococci within 1 or 2 minutes, atmospheres into which large excesses of glycerin or isosorbide had been heat-volatilized, exhibited no killing action whatever, even after 20 minutes. On the other hand, lactic acid is a compound which though equally water-soluble, has a vapor pressure appreciably higher than that of triethylene glycol. Hence, a concentration of lactic acid vapor higher than that of triethylene glycol would be required to attain the same per cent saturation and

TABLE II
Comparison of Physical Properties and Germicidal Action *in Vitro* of Four Compounds Which Differ Widely in Effectiveness as Aerial Disinfectants

	Vapor pressure (122°C.)*	H ₂ O solubility	Time necessary for a 70 per cent con- centration to kill 90 per cent of a standard suspension <i>in vitro</i> of hemolytic streptococci, Group C (3)
Isosorbide $ \begin{array}{ccccccc} & & & & \text{O} & & \\ & & & & / \quad \backslash & & \\ \text{H} & \text{H} & & & \text{H} & \text{H} & \\ & & & & & & \\ \text{HC} & -\text{C} & -\text{C} & -\text{C} & -\text{C} & -\text{CH}_2 & \dagger \\ & & & & & & \\ & & & & \text{OH} & \text{H} & \\ & & & & \backslash & / & \\ & & & & \text{O} & & \end{array} $	0.3(19)	∞	> 5 hrs.
Glycerin	0.84(18)	∞	5 hrs.
Triethylene glycol.....	1.61(20)	∞	4 min.
Lactic acid.....	15(21)	∞	< 1 min.

* In the absence of complete vapor pressure data at room temperatures, their volatilities are compared by means of their vapor pressures at 122°C.

† We are indebted to the Atlas Powder Co., Wilmington, Delaware, for supplying the samples of isosorbide.

hence the same extent of condensation on the bacteria-containing droplets. However, the increased germicidal efficiency of the lactic acid molecules enables them to operate in a smaller concentration. Thus, it is not surprising that this compound is effective in the air at levels equal to or less than those required for triethylene glycol (22).

In this discussion, it has been assumed that the killing action exerted on air-borne bacteria by a germicide condensed upon them proceeds by the same enzymic mechanisms by which the bacteria are killed by this agent *in vitro*. Experiments have been performed testing this point, and they will be described in detail in a forthcoming publication (23). It may be stated here, however, that the concentration of any germicide required to produce a given lethal action inside an air-borne bacterial droplet is similar to though slightly smaller than that which is necessary to produce the same effect *in vitro*.

7. Effect of Temperature Changes at Constant Relative Humidity

The influence of temperature changes on chemical aerial disinfection may be predicted directly from the foregoing discussion. Rise of temperature has two principal effects on the phenomena we are considering. It increases the vapor pressure of all compounds, and also hastens the rate of the toxic action of most bactericidal agents. If for any compound the magnitude of the first of these effects is greater than that of the second, raising the temperature will lower the bactericidal efficiency of any given absolute concentration of the vapor. Experiments with glycols bearing out this behavior have already been described (8, 12).

On the other hand, if the temperature is raised and, at the same time, more vapor is introduced into the air so that the per cent saturation is kept constant, the killing action will be augmented. Under these conditions, the concentration of germicide within the droplet remains constant, but its lethal action is increased at the higher temperature. Use of glycolstat control (24) makes it possible to compensate automatically for temperature changes, so as always to maintain a constant per cent saturation of the air. Experimental studies under such conditions are now being conducted, and will be presented in a forthcoming paper (25).

DISCUSSION

Experimental results which are consistent with the thesis that aerial disinfectants operate through the medium of the vapor phase have been reported by several investigators (12, 14, 26). Those of Baker and Twort (27) are worthy of mention. Recently they repeated our demonstration (28, 29) that pure vapors produced by evaporation from exposed liquid surfaces are rapidly lethal toward air-borne bacteria, and succeeded in obtaining excellent results with compounds like phenol, and chlorthymol for which their earlier reports had claimed activity only when dispersed as aerosols, but not as vapors (30).

The "germicidal aerosol" theory was proposed to explain the observation, reported by several earlier investigators, that killing of air-suspended bacteria does not occur unless the atmospheric concentration of bactericide exceeds its saturation point. A plausible explanation for this observation, which is sharply in divergence from the results reported here may be found in the following considerations: the experiments which gave rise to the "germicidal aerosol" theory, dealt largely with water-insoluble substances, mostly phenolic derivatives (17). We have shown how such compounds, having little affinity for water, would not exhibit much tendency to condense until a concentration near or equal to the saturation point had been achieved in the atmosphere. Moreover, most of the compounds employed by these earlier workers possessed extremely low vapor pressures. Thus, the difference between the concentration required for bactericidal action and the saturation point represents so small an

amount of material that it might easily have escaped detection by the methods employed. This appears likely in view of the fact that the analytical procedures used to determine the concentrations of effective material in the atmosphere were often admittedly highly approximate (17). In many experiments these concentrations were simply estimated on the basis of the loss in weight of liquid solution vaporized, and the volume of the chamber into which it was introduced. We have found that even in very tightly sealed chambers, as much as one-half of the weight of a compound which is volatilized in this way, may be rapidly removed from the air by adsorption on wall surfaces (2). Thus, it is possible that the effective concentrations prevailing in these earlier experiments, were significantly less than the reported values.

In this paper the laws of solution have been applied to calculate the concentration of germicide which collects about bacteria suspended in moist liquid droplets, under various conditions of temperature, humidity, etc. We have obtained equations which would be applicable to any soluble aerial disinfectant, but we have not dealt with possible variations in the activity coefficients, γ_g , and γ_{H_2O} , which enter into these expressions. Raoult's law can be used to formulate a general picture of the operation of the various factors here considered. For any particular germicide, however, corrections must be applied for deviations from the laws of perfect solution, if results of more than qualitative significance are desired. In forthcoming papers, we shall apply these corrections for some specific compounds and shall compare the predicted results so obtained with quantitative experimental data on the killing efficiency of various per cent saturations of a germicide vapor at various relative humidities and temperatures. It is possible, by extension of the concepts here presented, to predict how the killing of air-borne microorganisms can be expedited through the use of mixtures containing more than one vapor. Such effects will also be considered in a future publication.

The present paper has dealt mainly with the conditions necessary for an aerial disinfectant to kill microorganisms efficiently. The additional properties which such a compound must possess, in order to be capable of application in spaces inhabited by human beings have been discussed elsewhere (31, 17).

SUMMARY

The effectiveness of any compound as an aerial germicide depends upon the extent of condensation of its vapor on air-suspended bacteria, and on the rate at which the resulting concentration of germicide can produce death of the microorganisms.

The properties of any compound conducive to production of the highest rate of kill of air-borne microorganisms by means of the smallest possible concentration of germicide vapor, are as follows: (a) a low vapor pressure, but not

lower than 0.001 mm. Hg at 25°C.; (b) high hygroscopicity; (c) toxicity for bacterial metabolism—a high degree of potency is not necessary although the killing action will be more efficient the higher the antibacterial activity of the compound employed.

For any compound the killing action is always a direct function of the concentration of its vapor in the air. The maximum amount of a hygroscopic substance which can exist in the vapor state decreases as the relative humidity increases. Hence, at high relative humidities the bactericidal efficiency is lowest. At lower relative humidities the air can contain more vapor, and hence a greater effect is possible. At any relative humidity, the killing action is greater, the more closely the germicide vapor concentration approaches the saturation point.

The presence of soluble compounds in droplets containing bacteria promotes more extensive condensation of the germicide than would otherwise occur, and so enhances its effectiveness.

In the absence of such soluble substances, low atmospheric humidities may cause complete desiccation of a bacterial particle. Under these conditions its surface may become resistant to the condensation of the vapor, and thus prevent effective germicidal action.

The influence of temperature changes on the killing efficiency may be correctly deduced from a consideration of the effect of a rise in temperature on the vapor pressure of the germicide and on the rate of its bactericidal action *in vitro*.

Equations are presented for estimating quantitatively the magnitude of some of the effects discussed.

BIBLIOGRAPHY

1. Puck, T. T., *J. Exp. Med.*, 1947, **85**, 729.
2. Robertson, O. H., Puck, T. T., and Wise, H., *J. Exp. Med.*, 1946, **84**, 559.
3. Robertson, O. H., Appel, E., Lemon, H. M., Puck, T. T., and Ritter, M. H., data to be published.
4. Puck, T. T., and Wise, H., *J. Physic. Chem.*, 1946, **50**, 329. Wise, H., and Puck, T. T., *Science*, in Press.
5. Finn, S. R., and Powell, E. O., *Nature*, 1939, **144**, 864.
6. Williamson, A. E., and Gotaas, H. B., *Ind. Med.*, 1942, **11**, *Ind. Hyg. Sect.* 3, 40.
7. Masterman, A. T., *J. Ind. Hyg. and Toxicol.*, 1938, **20**, 278.
8. Puck, T. T., Robertson, O. H., and Lemon, H. M., *J. Exp. Med.*, 1942, **78**, 387.
9. Robertson, O. H., Puck, T. T., Lemon, H. M., and Loosli, C. G., *Science*, 1943, **97**, 142.
10. Baker, A. H., and Twort, C. C., *J. Hyg.*, Cambridge, Eng., 1941, **41**, 117.
11. Wells, W. F., and Zappasodi, P., *Science*, 1942, **96**, 277.
12. DeOme, K. B., *et al.*, *Am. J. Hyg.*, 1944, **40**, 239.
13. Twort, C. C., and Baker, A. H., *J. Hyg.*, Cambridge, Eng., 1942, **42**, 266.

14. Personnel of Naval Laboratory Research Unit No. 1, *U. S. Nav. Med. Bull.*, 1944, **42**, 1288.
15. Houghton, H. G., *Bull. Am. Meteorol. Soc.*, 1938, **19**, 153.
16. Houghton, H. G., *Physics*, 1933, **4**, 419.
17. Twort, C. C., Baker, A. H., Finn, S. R., and Powell, E. O., *J. Hyg.*, Cambridge, Eng., 1940, **40**, 253.
18. Lawrie, J. W., *Glycerol and the Glycols*, New York, Chemical Catalog Co., 1928, 191.
19. Atlas Powder Co., personal communication.
20. Gallagher, A. F., and Hibbert, H., *J. Am. Chem. Soc.*, 1937, **59**, 2514.
21. Krafft, F., and Dyes, W. A., *Ber. chem. Ges.*, 1895, **28**, 2596.
22. Lovelock, J. E., Lidwell, O. M., and Raymond, W. F., *Nature*, 1944, **153**, 20.
23. Puck, T. T., and Dunklin, E., data to be published.
24. Puck, T. T., Wise, H., and Robertson, O. H., *J. Exp. Med.*, 1944, **80**, 377.
25. Robertson, O. H., Lester, W., Puck, T. T., Wise, H., and Lemon, H. M., data to be published.
26. Stone, J. D., and Burnet, F., *Australian J. Exp. Biol. and Med. Sc.*, 1945, **23**, 205.
27. Baker, A. H., and Twort, C. C., *J. Hyg.*, Cambridge, Eng., 1944, **43**, 382.
28. Robertson, O. H., Bigg, E., Miller, B. F., Baker, Z., and Puck, T. T., *Tr. Assn. Am. Physn.*, 1941, **56**, 353.
29. Robertson, O. H., Bigg, E., Puck, T. T., Miller, B. F., and Baker, Z., *Am. Assn. Advance. Sc.*, Pub. No. 17, 1941, 271.
30. Baker, A. H., Finn, S. R., and Twort, C. C., *J. Hyg.*, Cambridge, Eng., 1940, **40**, 560.
31. Robertson, O. H., *Harvey Lectures*, 1942, **38**, 227.
32. *Synthetic Organic Chemicals, Technical Data*, New York, Carbide and Carbon Chemicals Corporation, 1940.
33. *International Critical Tables*, New York, McGraw-Hill Book Company, 1928, **3**, 217.