pellet of satisfactory hardness depends on the length of the pellet, the diameter of the tube, and the material.

Calibration data are presented for two implantors of each size (Table 1); pellets made of cholesterol were weighed individually on a microbalance.

TABLE 1

| Implantor No. | Implantor Diam (mm) | Pellet length (mm) | Tampings (No.) | Sample size (*n*) | Mean wt (mg) | S.D. |
|---|---|---|---|---|---|---|
| 1 | 0.85 | 1.0 | 10 | 10 | 0.534 | ± 0.03 |
| | | 2.0 | 20 | 30 | 0.984 | ± .05 |
| 2 | 0.835 | 1.0 | 10 | 10 | 0.520 | ± .02 |
| | | 2.0 | 20 | 20 | 0.877 | ± .06 |
| 1 | 2.505 | 1.0 | 10 | 10 | 4.896 | ± .4 |
| | | 2.0 | 20 | 10 | 8.345 | ± .26 |
| | | 3.0 | 25 | 10 | 12.585 | ± .5 |
| 2 | 2.48 | 1.0 | 10 | 10 | 4.483 | ± .4 |
| | | 2.0 | 20 | 10 | 8.705 | ± .5 |
| | | 3.0 | 25 | 10 | 12.117 | ± 0.4 |

By tapering the tube end to a cutting edge, the device can be used as a punch and implantor for thin slices of sterile tissue.

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## Bactericidal Effects of Propylene and Triethylene Glycol Vapors on Airborne *Escherichia coli*[1]


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In 1941 it was initially reported from this laboratory that the vapors of certain glycols had marked bactericidal effects upon airborne microorganisms (*1, 2*), and these reports were shortly followed by others describing the phenomenon in more detail (*3–9*). In these early reports it was stated that the killing of airborne bacteria was most rapid in the presence of concentrations of either propylene or triethylene glycol vapor that most nearly approached the saturation levels for these substances in the air and that at concentrations below this range the bactericidal effects were less apparent. Furthermore, it was noted that the lethal action of glycol vapors was less marked at relative humidities above 70%. A wide variety of microorganisms, dispersed into the air from broth cultures, was found to be susceptible to the germicidal activity of the glycol vapors. Included among these were *Staphylococcus albus* and *aureus*; *Streptococcus hemolyticus,* Groups A and C; *Str. viridans*; Types I and III pneumococci; *Bacillus subtilis*; *Hemophilus influenzae* and *pertussis*; Friedlander's bacillus; and *Escherichia coli.* Subsequent investigations reported from this laboratory dealing with the mechanism (*10*), rate of bactericidal action of glycol vapors (*11*), and the effects of desiccation (*12*) have utilized Group C hemolytic streptococci, pneumococci, and staphylococci because these organisms were considered to be more typically representative of the respiratory pathogens. From this work has arisen a better understanding of the paramount significance of relative humidity and glycol vapor concentration in determining the bactericidal efficacy of these compounds in the air.

Independent investigators have reported data confirming the susceptibility of some of the previously listed organisms to the glycol vapors (*13*) and, in addition, have found that *Serratia marcescens* and *Salmonella pullorum* (*14*), *Penicillium notatum* (*15*), and tubercle bacilli (*16*) were killed when atomized into saturated atmospheres of these substances. In no instances were airborne vegetative microorganisms, atomized from broth cultures, found to be resistant to the germicidal action of the glycol vapors.

A recent report by Nagy and Mouromseff (*17*) has questioned the validity of these data and thrown doubt upon the lethal effect of propylene and triethylene glycol vapor upon airborne *E. coli.* Using this organism atomized from broth cultures into air containing unmeasured concentrations of either propylene or triethylene glycol, they were unable to demonstrate any bactericidal activity. From their data these authors concluded that the primary effect of the glycol vapors was to increase the sedimentation velocity of the airborne bacterial particles by condensing upon them and hence mechanically removing them from the air. Furthermore, they suggested that the organisms in the sedimented bacterial particles were not necessarily killed but, following evaporation of the condensed glycol, might be redispersed into the air and again be capable of initiating infection. Because their conclusions are so widely at variance with those of other workers in the field, as well as with our own, we considered it essential to report our data, which we believe demonstrate that, under the prescribed physical conditions, propylene and triethylene glycol vapors in adequate concentrations in the air are bactericidal for *E. coli* atomized from liquid cultures, as they are for other microorganisms.

The experiments included in this report were done in the 640-cu ft chamber which has been previously described in detail (*18*). A 17-in. fan, rotating at 500 rpm, was centrally located on the floor and the air stream directed against the ceiling, thus providing adequate turbulence for uniform mixing of the chamber air. Temperature and humidity were pre-

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cisely controlled, and the values were checked at the start and finish of each experimental run. All experiments were done at 70°–73° F.

The centrifugated sediments of young actively growing cultures of *E. coli* were resuspended in sterile heart infusion broth to make a fivefold concentration of the original cultures. This bacterial suspension containing $1.5 \times 10^9$ microorganisms/ml was dispersed from a Graeser atomizer placed above the fan and operated for 30 sec at a pressure of 100 mm Hg, utilizing dry air which had been passed through an activated alumina drying tower (Lectrodryer). The weight of the suspension atomized was $0.35 \pm 0.01$ g for the experiments reported, and the resulting bacterial aerosol had a bacterial median diameter of 1.5 μ (*19*).[3]

Following the completion of the control run, the humidity was adjusted to the desired level, and the triethylene glycol vaporizer set on the floor immediately below the circulating fan. Vaporization, accomplished by means of a 60-w light bulb immersed in a beaker of glycol placed inside a brass cylinder open at the top, was continued for several hours until a dense Tyndall beam was demonstrable. Air samples, taken at the beginning and end of each test experiment, were analyzed for triethylene glycol content by the method of Kayé (*20*). Concentrations ranging from 10 to 25 μg/l of air were found (equivalent to 2–10 times saturation). At the conclusion of each test the chamber was thoroughly washed and aired. Air analyses confirmed the absence of measurable concentrations of triethlyene glycol in the chamber during the control experiments.

Propylene glycol was vaporized from a beaker which was filled with three times the quantity of glycol calculated to saturate the air of the chamber and then was placed upon an electrically operated hot plate. Vaporization was continued for about 30 min until the maximum Tyndall effect was noted, and then the test experiment was begun. Analytical determinations of the propylene glycol content of the chamber air taken during the test experiments and done according to the technique of Puck and Wise (*21*) revealed concentrations ranging from 0.2 to 0.4 mg of propylene glycol per liter of air (equivalent to 1–2 times saturation). At the conclusion of the experiment the chamber was thoroughly washed and ventilated, and analyses taken during the control experiments confirmed the absence of measurable concentrations of propylene glycol in the chamber air.

Two methods of bacterial sampling were used, one of which, the Folin-bubbler sampler (*22*), was most effective for fine bacterial particles (less than 5 μ in diam), and the other, consisting of 3% agar settling plates, was selective for larger particles. By the use of two such diverse methods of bacterial sampling it was felt that it would be possible to follow quantitatively the behavior of the fine bacterial particles capable of long airborne carriage, as well as the sedimentation of the larger droplets that were present in the air for relatively short periods of time following atomization. The bubbler samplers were operated at a flow of 1 cu ft air/min according to the schedule shown in Tables 1 and 2. Appropriate aliquots were then taken from the bubbler samplers and plated in 3% agar, incubated for 18 hr, and the colonies counted. From this data it was possible to calculate the number of viable organisms per cu ft of air recovered by the sampler.

[3] This value indicates that 50% of the viable bacteria were contained in particles of less than 1.5 μ.

TABLE 1

EFFECT OF PROPYLENE GLYCOL VAPOR UPON AIRBORNE *E. coli* AT 72° F

| Sampling (time interval from beginning of spray in min) | No. of viable organisms recovered | | | |
|---|---|---|---|---|
| | Control (no glycol) | | Test (glycol 11.0 mg/ft³) | |
| | Bubbler No./ft³ of air | Settling plate | Bubbler No./ft³ of air | Settling plate |
| *A*, Relative humidity = 70% | | | | |
| 0– 5 | 4180 | 459 | 28 | 211 |
| 1– 6 | 4120 | 499 | 12 | 147 |
| 2– 7 | 3720 | 427 | 16 | 80 |
| 3– 8 | 3300 | 443 | 4 | 36 |
| 4– 9 | 2540 | 284 | 3 | 21 |
| 6–11 | 1900 | 262 | 0.8 | 6 |
| 10–15 | 2840 | 246 | 0.8 | 0 |
| 15–20 | 2160 | 240 | 0 | 0 |
| | $K_B$* = 0.025 | $K_{SP}$† = 0.025 | $K_B$ = 0.27 | $K_{SP}$ = 0.29 |
| *B*, Relative humidity = 50% | | | | |
| | Control (no glycol) | | Test (glycol 14.7 mg/ft³) | |
| 0– 5 | 2440 | 223 | 0 | 7 |
| 1– 6 | 3640 | 192 | 0 | 1 |
| 2– 7 | 1760 | 164 | 0 | 0 |
| 3– 8 | 1800 | 148 | 0 | 0 |
| 4– 9 | 2100 | 133 | 0 | 0 |
| 6–11 | 1680 | 131 | 0 | 0 |
| 10–15 | 1520 | 89 | 0 | 0 |
| 15–20 | 1140 | 70 | 0 | 0 |
| | $K_B$ = 0.022 | $K_{SP}$ = 0.029 | $K_B = \infty$ | $K_{SP}$ = 1.71 |

* $K_B = K_{\text{Bubbler}}$.
† $K_{SP} = K_{\text{Settling plate}}$.

The open settling plates were exposed for 5-min periods at the same time and on the same schedule as was used for the bubbler samplers and shown again in Tables 1 and 2. Following exposure the settling plates were incubated for 18 hr, and the resultant colonies counted.

The data presented in Tables 1 and 2 are typical of the results obtained when *E. coli* was atomized from liquid cultures into saturated atmospheres of either propylene or triethylene glycol vapor at various relative humidities. These particular experiments were selected from many similar ones for illustrative purposes. They demonstrate the marked dependence of the bactericidal action of the glycol vapors upon the relative humidity, with decreasing rates of kill occurring as the humidities were raised above 50%. By plotting the numbers of survivors logarithmically

as a function of time, the data for both the bubbler samplers and the settling plates fell on a straight line. Using the slopes of these lines as indices of the rates of kill, die-away constants were obtained which permitted comparison between experiments and were designated $K$ values.[4]

The analytical values given for the amount of glycol present in the air are greater than the calculated quantities necessary to saturate the atmosphere with vapor because the analytical procedures employed sampled droplets of glycol as well as vapor. These droplets of glycol serve as excellent sources for glycol vapor to compensate for the loss of vapor due to condensation on the walls and floor (*23*) of the experimental room. Thus the maintenance of 100% saturation of the air by glycol vapors is assured for the duration of the experiment.

Only a few seconds are required for the glycol vapor to condense on the bacteria-containing particle and for this droplet to reach equilibrium with the atmosphere in which it is suspended (*10*). The amount of glycol vapor condensing on the particle will be determined by the relative humidity and the degree of saturation of the air by the vapor. In the light of these considerations it can be seen that, under the proper conditions, the effects of the glycols should become immediately apparent. Two possible effects might be conceived: the disappearance of viable cells due to a true bactericidal action comparable to that observed for the action of glycols *in vitro* (*24*) or by the sedimentation of the bacteria-containing droplets resulting from the gain in mass caused by the condensation of glycol vapor upon the airborne particles (*17*). Both effects will be dependent upon the relative humidity and the percentage saturation of the atmosphere but to a different order of magnitude.

TABLE 2

EFFECT OF TRIETHYLENE GLYCOL VAPOR UPON AIRBORNE *E. coli* AT 72° F

| Sampling (time interval from beginning of spray in min) | No. of viable organisms recovered | | | |
|---|---|---|---|---|
| | Control (no glycol) | | Test (glycol 14.7 γ/l) | |
| | Bubbler No./ft³ of air | Settling plate | Bubbler No./ft³ of air | Settling plate |
| *A*, Relative humidity = 10% | | | | |
| 0– 5 | 4640 | 284 | 760 | 140 |
| 1– 6 | 7520 | 272 | 560 | 102 |
| 2– 7 | 6640 | 248 | 544 | 43 |
| 3– 8 | 5120 | 212 | 112 | 22 |
| 4– 9 | 6440 | 172 | 123 | 13 |
| 6–11 | 5280 | 148 | 21 | 6 |
| 10–15 | 5560 | 140 | 2 | 8 |
| 15–20 | 2240 | 109 | 0 | 0 |
| | $K_B = 0.021$ | $K_{SP} = 0.027$ | $K_B = 0.288$ | $K_{SP} = 0.247$ |
| *B*, Relative humidity = 70% | | | | |
| | Control (no glycol) | | Test (glycol 25.8 γ/l) | |
| 0– 5 | 4740 | 608 | 2332 | 496 |
| 1– 6 | 5000 | 540 | 2952 | 460 |
| 2– 7 | 4240 | 416 | 816 | 360 |
| 3– 8 | 4480 | 464 | 432 | 284 |
| 4– 9 | 3920 | 531 | 621 | 320 |
| 6–11 | 2700 | 423 | 280 | 351 |
| 10–15 | 2180 | 417 | 246 | 347 |
| 15–20 | 2040 | 319 | 73 | 275 |
| | $K_B = 0.039$ | $K_{SP} = 0.015$ | $K_B = 0.082$ | $K_{SP} = 0.012$ |

[4] $K = \frac{\text{Log } N_o - \text{log } N_t}{t}$.

$K$ = Die-away constant.
$N_o$ = Numbers of survivors at time zero.
$N_t$ = Numbers of survivors at time $t$ (in minutes).

The bactericidal nature of the glycols *in vitro* can be easily demonstrated, and such action has been shown to be very dependent upon the concentration of these substances surrounding the bacterial cells. At certain levels, a change of 10% in the glycol concentration results in a 100- to 1000-fold change in the death rate (*24*). This sensitivity of the rate of bactericidal action of the glycols to minute changes in their concentration is further demonstrated in the data secured from aerosol studies (*11*). Such small changes in the concentration of glycol vapor in the air, and hence in the droplet, cannot alter the sedimentation rate by the same order of magnitude. Calculations based upon the Stokes' equation for the velocity of turbulent settling of spherical particles show that a 10- to 35-fold increase in diameter (or a $10^3$–$10^5$ increase in mass) would be required to give the same change in the apparent death rate of *E. coli* as that obtained in lowering the relative humidity from 70 to 50% (Table 1). Thus, both *in vitro* and in the air, the rate at which the bactericidal activity varies with changes in relative humidity or glycol concentration is much higher than the rate of change in settling velocity. Therefore, from these considerations, also, it is evident that the observed rates of action of the glycols on airborne *E. coli* are consistent with the bactericidal action of these agents and cannot be accounted for on the basis of sedimentation.

There are several other criteria for establishing the mode of action of the glycols. If the action is primarily due to settling, as claimed by Nagy and Mouromseff, then the decrease in the viable population of the air should give rise to an increase in the numbers of viable organisms recovered on the initial settling plates of the test experiment. The higher the rate of settling, the greater will be the number of organisms recovered on the first test settling plate. This possibility is clearly eliminated by the data shown in Tables 1 and 2, which are typical of the results obtained in more than fifty experiments. Not one instance was found in which any test settling plate gave a higher viable count than was obtained for the control plate for the same time interval.

Further, the rate of removal of bacteria from the air by settling should be independent of the type of organism studied, whereas it is generally accepted that the rate at which any bactericidal agent acts is influenced by the type of organism tested. It has been demonstrated that the bactericidal activity of the glycol vapors is dependent upon the type of organism

against which they are being employed, both in the air (*6, 11*) and *in vitro* (*24*). Finally, direct evidence of the lethal action of glycol vapor was obtained by testing the viability of microorganisms isolated by sedimentation from propylene glycol saturated atmospheres, and in no instances were any viable bacteria found (*4*).

Since it has been found that the rate of bactericidal action of glycol vapors depends directly upon the concentration of glycol in the air (Puck [*10*]), we could infer that the observed lack of lethal effect of propylene and triethylene glycol vapor for *E. coli* reported by Nagy and Mouromseff was due to insufficient glycol in their experiments. They report no determinations of glycol in the air. As Robertson (*23*), Lester *et al.* (*25*), and Bourdillon (*13*) have shown, considerable quantities of glycol vapor are lost by condensation on walls and other surfaces of the room, so that evaporation of a quantity of glycol calculated to produce saturation of a given space may result in only an insignificant fraction of such concentration. Furthermore, as was shown by Puck and Chaney (*26*), the presence of a visible fog is never in itself sufficient to prove the existence of a saturated atmosphere of glycol vapor. Hence no valid conclusions about the effectiveness of the glycols or any other bactericidal vapors can be drawn without knowledge of the concentration employed. In our experiments, conducted under optimum conditions of temperature and humidity, and employing atmospheres known to be saturated with propylene glycol, the killing of airborne *E. coli* was essentially instantaneous. Triethylene glycol acted more slowly.

Triethylene glycol vapor under similar conditions was found to be highly bactericidal against airborne *E. coli*. However, the rate of kill was demonstrably less than that observed for propylene glycol. This difference conforms to the results of *in vitro* studies (*24*) which demonstrated that, of the two agents, propylene glycol is the more rapidly acting germicide.

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# *Comments and Communications*

## On Leukotaxine

As pointed out in numerous publications, inflammation is a manifestation of severe cellular injury in vertebrates (*1*). It has also been pointed out that any irritant injures the cell, and injury alters the biochemistry of the cell, with consequent release of various common denominators (*1*, p. 135, footnote). These common denominators include leukotaxine, the leukocytosis-promoting factor (thermolabile and thermostable), necrosin, pyrexin, and leukopenic factor, leukopenin, and the more recently described exudin (*2*). These factors, or common denominators, are responsible for the fundamental, stereotyped reaction of inflammation. It is incorrect to state that one of them alone, and not all of them or some of them, initiate acute inflammation, as Moon asserts that the writer claims (*3*).

It has often been pointed out that the cells, when injured, liberate leukotaxine, and only when injured do they form this substance. Even a diagram of this process appears in a recent monograph (*1*, p. 123). It would be difficult, therefore, to envisage that normal tissue cells as such would contain leukotaxine. In recent studies Moon has questioned the hypothesis on the ground that leukotaxine has not been shown to exist in normal tissue (*3*). One would not expect to find it in perfectly normal cells. Moon presents absolutely no evidence supporting the view that leukotaxine does not exist in normal tissue (*3*). His own studies indicate that a chemotactic substance is possibly present in damaged tissue; and this substance does not necessarily come from exudate where leukotaxine had originally been recovered. Moon obtained a quantity of muscle from a freshly killed rabbit and ground the material in a meat chopper. A saline extract was then made. Similar extracts were made from kidney, liver, skin, and lung. These extracts were then injected intradermally into the ears of rabbits, and