Effect of Propylene and Triethylene Glycol on Atomized *E. coli*

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The use of propylene and triethylene glycol as an air disinfectant has received considerable publicity in recent years. Twort *et al.* (*15*) have made an extensive survey of the efficacy of various germicides dissolved in glycol solvents, in destroying airborne organisms. However, these authors did not report any bactericidal effect by glycols per se. Robertson *et al.* (*13*) reported that propylene glycol as an aerosol possessed marked germicidal properties. Robertson *et al.* (*12*) and Puck *et al.* (*8*) believed that propylene glycol vapor destroyed certain respiratory pathogens when 1 g of the vapor was present in 50,000,000 ml of air. Robertson *et al.* (*14*) also stated that the virus of influenza could be inactivated by glycol vapor in a 1:3,000,000 dilution.

In discussing the mechanism of glycol disinfection, Puck (*5*) attributed the killing action to the high concentration of glycol as a result of condensation of the vapors onto the droplets containing the organisms. He assumed that in about 20 sec the droplets condensed 20 times as much glycol as the weight of the original particle. As shown by Wells (*16*), the rate of fall of such a particle would be greatly accelerated, and it would appear that the removal of the organisms from the air by means of glycol could be accounted for by a mechanical process.

Hamburger *et al.* (*1*) and Puck *et al.* (*7*) showed that glycol vapors were not effective against dustborne organisms. Puck (*5*) stated that the killing action is always a direct function of concentration of glycol vapor in air. The maximum effect is reported by Robertson (*11*) to be at about 60% relative humidity with the vapor at the saturation point. Under these conditions small variations in temperature will result in a fog and condensation on the walls. As the concentration of glycol is diminished below saturation, the apparent bactericidal action is diminished. Other difficulties in the practical use of glycols have been summarized by the Committee on Sanitary Engineering, National Research Council, Division of Medical Science (*17*). The physical conditions under which the organisms are removed from the air appear to be very critical. It would seem that further study is required to elucidate the mechanism of glycol action and its effectiveness in air sterilization. The present paper reports the results of some experiments made to determine whether the removal of bacteria from the air, by vapors of triethylene glycol, is lethal or merely mechanical.

Experimental chamber tests. The experimental chamber was a box 4 ft on each side, as described by Rentschler and Nagy (*9*). A specially constructed drawer in the bottom of the box was so arranged that a number of sterile Petri plates could be exposed for various periods of time after spraying the organisms into various types of atmospheres with or without glycol. *Escherichia coli* was used as the test organism for all the experiments. Simultaneously with the plates, bacteria were collected with a Luckiesh-Holladay-Taylor (*3*) electrostatic precipitator.

A dilution of 0.1 ml of a 24-hr culture of *E. coli* was made in 20 ml of broth and this bacterial suspension was sprayed into the chamber using a Devilbiss spray gun at an air pressure of 50 psi so as to produce a fine spray. After 30-sec settling of the spray to remove the very large droplets, both the Petri plates and precipitator were used to collect the organisms. The Petri plates were exposed for only 2 min, so there would not be too many colonies per plate. The plates in the precipitator were changed every 5 min until 4 sets were collected. All the plates were incubated for 24 hr at 37° C and the colonies were counted. The results of these tests are given in Table 1.

TABLE 1

COMPARISON OF THE PLATE COUNTS OF *E. coli* SPRAYED INTO VARIOUS ATMOSPHERES OF A TEST CHAMBER

Test No.	Type of atmosphere	Relative humidity, %	No. of *E. coli* settling on plates in 2 min	Plate count from precipitator				
				1st 5 min	2nd 5 min	3rd 5 min	4th 5 min	Total
1	*E. coli* in air	50	2,800	10,000	1,890	2,450	1,450	15,890
2	Propylene glycol aerosol 1 g–1,700,000 ml of air followed by *E. coli*	65	3,400	11,300	1,750	1,300	835	15,225
3	Propylene glycol 1 g–1,700,000 ml of air sprayed simultaneously with *E. coli*	75	4,000	12,000	1,950	1,460	1,070	16,730
4	Air saturated with propylene glycol vapor followed by *E. coli*	73	3,400	9,700	5,400	2,020	330	17,450
5	Water vapor followed by *E. coli*	95	2,800	10,000	1,460	430	205	12,095

These results indicate that the organisms are removed from the air more rapidly with glycol than when no glycol is present. However, the total number of organisms settling on the plates is of the same order of magnitude as the controls without glycol, showing that the bacteria are not destroyed by glycol. The findings are consistent in that settling on Petri plates gave figures similar to those obtained with the precipitator. In those tests with

TABLE 2

TOTAL NUMBER OF *E. coli* COLLECTED ON PETRI PLATES IN A SCHOOLROOM WITH AND WITHOUT TRIETHYLENE GLYCOL VAPORS

Without glycol (control)			With glycol			Relative humidity, %	Temperature in °F
Test	On plates	With precipitator	Test	On plates	With precipitator		
A 1*	8,171	2,800	A 3	11,540	10,000	47	70°
2	9,900	4,000	4	12,600	10,000		
5	8,490	10,000					
B 1	4,865	2,700	B 3	9,530	5,000	46	70°
2	6,570		4	12,090			
5	9,640	6,000					
C 1†	6,080	6,000	C 5†	6,645	5,600	50	75°
2	5,120	3,500	6	6,250	5,600		
3	1,065	1,750	7	3,120	2,200		
4	315	290	8	1,950			

* Sampling of the bacteria was in the order numbered.
† Sprayer turned off and samples collected every 15 min.

glycol the amount used was more than enough to saturate the atmosphere of the box. The effect of glycol on the bacteria apparently is the same as that of saturated air; namely, the droplets do not evaporate but settle rapidly, as seen in Test 5.

School tests. A local schoolroom was available to us for various epidemiological studies. During the absence of children, a commercial triethylene glycol vaporizer was installed in the room and tested for its efficacy in destroying *E. coli.* The vaporizer was adjustable so that different amounts of triethylene glycol vapor could be introduced into the air. The infector was an Arnold vaporizer emitting a constant fine spray of *E. coli,* suspended in dilute broth. The bacterial sprayer was in operation throughout an entire series of tests. Sterile Petri plates located at various points in the schoolroom were used as collectors. In addition, samples were also collected by means of a Luckiesh-Holladay-Taylor precipitator. Sampling of the air for each test was done for 20 min. When glycol was used, the vaporizer was on for an hour before the bacterial sprayer was turned on. This was to insure that the amount of glycol in the air was near saturation. The results of a series of tests are shown in Table 2.

The number of organisms collected by the Petri plates and the precipitator was greater when glycol was present than on the controls without glycol. This again shows that glycol vapors increase the rate of settling of droplets but that the organisms are not destroyed. This is especially evident in Test C, Table 2. In both cases (i.e., with and without glycol), the room was infected to approximately the same degree, but the number of organisms collected when glycol was present was relatively much greater.

Duct and room tests. A room 20 ft square and 11 ft high was equipped with a blower delivering approximately 600 cu ft of air/min, and with a duct so as to distribute the air evenly. The type of circulating system is very similar to that found in an office or home. An Arnold vaporizer at the entrance to the duct was used to contaminate the air with *E. coli.* A triethylene glycol vaporizer was placed about 8 ft inside the duct. All the bacteria entering the duct had to pass through the glycol vapors before emerging into the room. In the second part of the experiment the glycol vaporizer was placed in the center of the room. The air was sampled for 20 min by means of Petri plates located in various positions. Results of these experiments are shown in Table 3.

TABLE 3

TOTAL NUMBER OF *E. coli* COLLECTED ON PETRI PLATES IN ROOM WITH AND WITHOUT TRIETHYLENE GLYCOL

Test No.	Control	Glycol	Relative humidity, %	Temp. in °F	Position of vaporizer
1	5,830	5,940	50	70	In duct
	5,510	5,600			In "
2	279	311	35	70	In "
3	691	712	40	75	In room
4	292	370	45	70	In "
5	6,350	6,700	40	70	In "
	6,450	7,050			

The total number of bacteria settling on the Petri plates, whether or not triethylene glycol is present in the air, is within the limits of experimental error. Having the triethylene glycol vaporizer in the duct would appear to be the ideal position, according to Puck and Chaney (*6*), if the vapor were germicidal. All the organisms were intimately mixed with the vapors under these conditions. The vapors were not condensed on the side of the duct because the duct was very short; glycol was definitely detectable in the room. When the same amount of glycol that was sufficient to saturate the air was evaporated directly in the room, the results once more indicated that the vapors were not germicidal. The plate count with glycol in the air was again slightly higher, showing the mechanical removal of the organisms by glycol.

The results presented here show that glycols do not destroy bacteria. This is contrary to the results reported by Robertson and his collaborators. It would appear that there are some fundamental differences in technique or interpretation of data to account for such a discrepancy in results. Our present methods of spraying and

sampling *E. coli* have been effective in the collecting of organisms in air ducts, rooms, and test boxes over a period of 11 years (Rentschler *et al.* [*9*]). Settling of organisms on Petri plates has been shown to give reproducible results. This has also been demonstrated by Robertson *et al.* (*10*). To obviate the effect of air currents, as many as 10 Petri plates, placed in various positions in a room, were used for one test. In the experiments described, Petri plates, the Hollaender Dallavalle sampler, and the Luckiesh-Holladay-Taylor electrostatic precipitator placed at the bottom of the test box gave similar results. This indicates that sampling could not account for the variance in the figures of Robertson *et al.* and those in our work.

The amount of glycol in the air could not have been a factor in our tests. Both propylene and triethylene glycol were calculated to give a saturated atmosphere, and in the case of a commercial vaporizer the vapors were visible in the room. According to Puck (*5*), the killing is always a direct function of the concentration of glycol in the air. It would be expected, therefore, that some indication of bactericidal effect would be seen even when conditions were not optimum. However, the tests indicate that the glycols increased the rate of fall of the organisms and showed no germicidal action. Twort used propylene glycol with hexylresorcinol but did not observe any bactericidal effect of the glycol per se. Mallmann and Churchill (*4*) found that spores of bacteria and of *Aspergillus niger* and *Penicillium italicum* were unaffected by glycol vapors or sprays. They concluded that these compounds were not effective in controlling microbial contamination of cold storage and food preparation rooms.

The apparent germicidal, viruscidal effect of glycol can be attributed to the precipitating of water droplets from air containing bacteria or viruses.

Puck (*5*) calculated that the weight of a droplet increases 20 times by adsorption of glycol vapors. According to Stokes' law,

$$v = \frac{2gr^2(d_1-d_2)}{9n},$$

where v = velocity of fall in cm/sec, g = gravity 980, r = radius of particles, n = coefficient of viscosity, d_1 = density of sphere, and d_2 = density of medium. The velocity of fall will be proportional to the square of the radius. If we take Robertson's (*12*) value of 3 μ as the diameter of a droplet, and the height of his experimental chamber as 15 in., it would take approximately 20 min for the organisms to settle. However, if the droplet increases in weight by 20 times and the diameter by three times, the rate of fall will be increased as the square of the radius so that the droplets will settle in 2.5 min. This is the value reported by Robertson (*11*) for the apparent germicidal and viruscidal action of glycols in his chamber. The Hollaender-Dallavalle (*2*) sampler in his apparatus would not have collected any more organisms after 2.5 min, whereas settling on Petri plates would have shown that all the organisms were precipitated, as in our tests. Using a larger box and the same size droplet, the length of time of settling would be proportional to the height, which accounts for the much longer settling time in our tests. The vapors do not condense on dust-borne organisms, and therefore the particles will remain suspended in air. It is conceivable that under practical conditions the glycol-precipitated organisms may lose their moisture and act as a reservoir for the reinfection of the air.

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Spade-Foot Toad Sperm as an Activating Agent in Producing Gynogenetic Haploid Embryos from *Rana* and *Pseudacris* Eggs

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Much interest has been directed toward the study of the mode of development of haploid frog embryos by experimental embryologists and geneticists in their search for a better understanding of nucleocytoplasmic relationships. Gynogenetic haploid embryos, resulting from eggs activated by the penetration of spermatozoa and developing with egg-chromatin alone, have been produced by one of two methods: (*a*) eggs inseminated by sperm which had been moderately irradiated beforehand (*1, 2, 3*) or had been treated with certain chemicals (*4, 5*); or (*b*) by the use of foreign sperm as found by G. Hertwig (*6*) and Tchou (*7*) in their hybridization experiments with certain European species of anurans.

Realizing the convenience of this latter method, the writers have applied it in crossing a number of species of anurans occurring in the United States. We found that, when eggs of *Rana pipiens* and *Pseudacris nigrita triseriata* were inseminated with sperm of the spade-foot toad, *Scaphiopus holbrookii holbrookii*, the embryos ob-

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