SPECIAL ARTICLES

STERILIZATION OF AIR BY CERTAIN GLYCOLS EMPLOYED AS AEROSOLS

THE possibility of sterilizing air by means of germicidal mists or aerosols was reported in 1938 by Trillat,[1] who has been investigating this subject for a number of years. Two groups of workers in England, Pulvertaft and Walker[2] and Twort, Baker, Finn and Powell[3] have since confirmed and extended Trillat's findings. Liquid aerosols consist of droplets of approximately 1–2 μ in diameter dispersed in the air. Larger particles tend to settle out quickly. The theoretical basis for the high bactericidal activity of germicidal aerosols is, briefly, as follows: a very small quantity of a germicidal agent may be dispersed throughout a relatively large enclosed air space, yet, *because each droplet of the aerosol contains the same concentration of the effective chemical substance as does the parent solution,* the bactericidal agent is enabled to act in high concentration on bacteria suspended in air. The actual amounts of the compound in the atmosphere may be of the order of one part by weight of aerosol to many million volumes of air.

The germicidal agents found to be most effective by the English workers were resorcinol and hexyl resorcinol. They have made tests on the oral toxicity of these compounds for animals, and find that in the amounts employed for air sterilization they are harmless for the animal body. However, both these substances possess a certain degree of toxicity, and insufficient data are available on local or systemic effects of breathing resorcinols over very long periods of time.

We became interested in the possibility of using as germicidal aerosols compounds with marked wetting and spreading properties such as the anti-bacterial detergents described by Miller and Baker.[4] Preliminary experiments indicated limited aerosol activity of aqueous solutions of such compounds. 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 related glycols themselves acted as effective bactericidal aerosols.[5] Because of the extremely low toxicity of propylene glycol,[6] and the advantage of employing a simple aerosol, consisting of only one compound, we have conducted most of our experiments with propylene glycol and a few very closely related substances, *e.g.,* trimethylene glycol and ethylene glycol.

The technique in our experiments was as follows: known quantities of a bacterial suspension were sprayed into a rectangular glass-walled chamber, of 60 liters cubic capacity. The air was gently agitated by means of a slowly rotating rubber-bladed fan. The number of viable bacteria recoverable from the chamber air was determined by withdrawing a measured volume of air at a constant rate through a glass funnel which was suspended directly above the surface of an agar plate within a sealed glass jar.[7] By this means a relatively uniform number of colonies was obtained following the introduction into the chamber of the same amount of standardized bacterial suspension. There is a progressive and quite constant diminution in the number of colonies recovered from successive air samples taken during the period of an hour. For this reason, two identical chambers were used for each experiment, one for the test and the other for the control. The germicidal aerosol was introduced into the chamber by means of a Graeser atomizer[8] which produces a mist sufficiently fine to act as an effective aerosol. The bacterial suspensions were usually sprayed into the chamber with this type of atomizer, although other atomizers producing coarser droplets were used in some experiments.

For experiments on the general principles of the method, *Staphylococcus albus* was employed as the test microorganism. It was found that one part by weight of propylene glycol in two million volumes of air, effected complete sterilization of an atmosphere containing as many as 200,000 bacteria per cubic liter of air. 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

TABLE I

THE EFFECT OF PROPYLENE GLYCOL AEROSOL ON STAPHYLOCOCCUS ALBUS

Time intervals of air samples	Number of colonies on plates from	
	Control chamber	Aerosol chamber
(1) Immediately after bacterial spray	2207	1860
(2) Immediately after H_2O in control and aerosol in test	764	1
(3) 15 minutes later	532	0
(4) 30 minutes later	336	0

[1] A. Trillat, *Bull. de L'Acad. de Med.,* 3 Serie, 119, 64, 1938.

[2] R. S. V. Pulvertaft and J. W. Walker, *Jour. Hygiene,* 39: 696, 1939.

[3] C. C. Twort, A. H. Baker, S. R. Finn and E. O. Powell, *Jour. Hygiene,* 40: 253, 1940.

[4] B. F. Miller and Z. Baker, SCIENCE, 91: 624, 1940.

[5] The English workers used glycols and glycerine as vehicles. However, as far as we can determine, they ascribed no importance to these compounds beyond their usefulness as hygroscopic solvents for the germicidal substances, resorcinol and hexyl resorcinol.

[6] P. S. Hanzlik, H. W. Newman, W. Van Winkle, A. J. Lehman and N. K. Kennedy, *Jour. Pharm. and Exp. Therapeut.,* 67: 101, 1939.

[7] This is a modification of the technique of air sampling described by Hollaender and Dallavalle. Public Health Reports, 54: 574, 1939.

[8] J. B. Graeser and A. H. Rowe, *Jour. Allergy,* 6: 415, 1935.

showed many hundreds of colonies. A protocol of this type of experiment is shown in Table I. Other microorganisms were found to be similarly susceptible to the action of this aerosol. Among those tested were pathogenic invaders of the respiratory tract, *e.g.*, pneumococci Type I and III, hemolytic streptococci and hemolytic staphylococci, as well as organisms of lesser or no pathogenicity, such as *Streptococcus viridans, B. coli* and *Micrococcus catarrhalis.* Bacteria sprayed into a chamber containing the germicidal mist were killed with equal rapidity.

Tests made with other glycols showed that ethylene glycol and trimethylene glycol were about as effective germicidal aerosols as propylene glycol. Glycerine, on the other hand, exhibited only slight killing action.

To prove that condensation of the glycol itself, on the collecting plates, does not inhibit growth of the organisms by bacteriostasis, suitable controls were performed which definitely ruled out any such "plate effect." Furthermore, it is conceivable that the reaction between the germicidal aerosol particles and the bacteria might somehow change the state of suspension of the bacterial droplets and prevent their adherence to the plates. To control this possibility, another method was employed for collecting the bacteria after exposure to the aerosol: air was drawn slowly through 25–50 cc of diluted nutrient broth in a glass cylinder containing many small beads. Plated samples of this fluid, through which air from the control chamber had been bubbled, yielded large numbers of colonies, whereas similar samples of fluid exposed to the aerosol-containing air were sterile.

The presence of killed bacteria in aerosol-treated air was demonstrated by condensing on a chilled microscope slide the moisture of the air drawn from the chamber. The microorganisms were stained and identified; samples of the condensed fluid showed no growth.

We have also eliminated the possibility that microorganisms, although rendered incapable of growth on artificial media, might nevertheless retain the capacity to reproduce in a suitable host. Experiments were conducted in which virulent pneumococci Type I were treated in the chamber with the propylene glycol aerosol. The air was then drawn through sterile broth in a bead tower, and 1 cc quantities of this fluid injected into mice. These animals survived. However, when the experiment was performed with air drawn from the control chamber, all the mice died of pneumococcic infection.

The only criterion employed heretofore for the lethal action of aerosols has been failure of the exposed organisms to grow on agar-coated surfaces. We believe that the more rigid types of experimental controls described above should be employed as additional methods of evaluating the germicidal activity of aerosols.

The mechanism of the aerosol action, the physical properties of the germicidal mists, the time duration of their effective action,[9] minimum effective concentration, activity of other compounds, etc., are at present under investigation. We are also observing the effect on the lungs and other body organs of animals breathing aerosol-containing atmospheres for extended periods of time.

O. H. ROBERTSON
EDWARD BIGG
BENJAMIN F. MILLER
ZELMA BAKER

UNIVERSITY OF CHICAGO

RACEMIZATION OF GLUTAMIC ACID WITH ALKALIES

FISCHER, Kropp and Stahlschmitt[1] racemized 1(+)glutamic acid by heating it in barium hydroxide solution in an autoclave at 160–170° for 9 hours. We have found that a barium hydroxide solution of 1(+) glutamic acid becomes optically inactive in about 88 hours when it is heated in an ordinary bacteriological autoclave at 120°.

25 g of 1(+)glutamic acid and 105 g of $Ba(OH)_2 \cdot 8H_2O$ were mixed with 500 cc of water. This mixture was heated intermittently in the autoclave. At intervals samples were removed, cooled to room temperature and filtered. 15 cc of the clear filtrate were mixed with 4 cc of concentrated hydrochloric acid, and the resulting solution was read in the polarimeter. The results obtained are plotted in the accompanying figure.

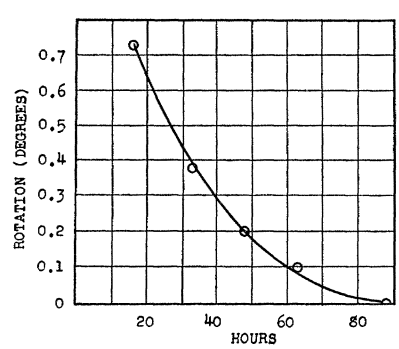

FIG. 1. Racemization of glutamic acid with barium hydroxide.

Town[2] mentioned using a sample of d,l-glutamic

[9] The germicidal action of the propylene glycol aerosol appears to be of brief duration. However, it has been found recently that the bactericidal effect may be prolonged for at least 90 minutes by the addition of a small quantity of glycerin.

[1] E. Fischer, W. Kropp and A. Stahlschmitt, *Ann. d. Chem.*, 365: 181, 1909.

[2] B. W. Town, *Nature*, 145: 312, 1940.