THE MECHANISM OF AERIAL DISINFECTION BY GLYCOLS AND OTHER CHEMICAL AGENTS

I. Demonstration that the Germicidal Action Occurs through the Agency of the Vapor Phase*

By THEODORE T. PUCK, Ph.D.

(From the Departments of Medicine and Biochemistry, The University of Chicago, Chicago)

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In a series of publications which have appeared during the last five years (1–8), the activity of certain organic compounds, particularly propylene and triethylene glycols, in killing air-borne bacteria and viruses has been described. The types of microorganisms found to be susceptible and some effects on this killing action of changes in the temperature, relative humidity, and concentration of the bactericidal agent, have been presented. An hypothesis was advanced to account for these observations, namely, that the lethal effect is due to condensation of vapor molecules of the active agent on to the bacteria-containing particles so that a bactericidal concentration of the germicide accumulates about the microorganisms.

Some of the evidence in support of this theory has been briefly described in preliminary reports from this laboratory (3, 4, 9), and additional confirmation has since been presented by other investigators (10–12). It is the purpose of the present paper to give a detailed account of the experimental evidence which demonstrated the validity of this mechanism. In subsequent publications some of the factors which govern the attainment of effective contact between molecules of the germicide and air-borne microorganisms will be developed (13) and studies on the metabolic character of the killing process by some specific aerial bactericides will be presented (14).

Killing of air-borne bacteria has been reported to occur through the action of a number of chemical agents, for example, phenol (15), NaOCl solutions (16, 17), resorcinol and *n*-hexyl resorcinol (18).[1] All of these compounds, however, are powerful bactericidal agents *in vitro*. The demonstration that propylene and triethylene glycols are effective in the air even in very great dilution revealed that it is not essential for an aerial disinfectant to be a powerful inhibitor

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[1] These and other investigations on chemical disinfection of the air have been recently reviewed (19, 20).

of bacterial metabolism. Propylene glycol, for example, will not produce rapid death of *Staphylococcus albus* in the test tube until a concentration of about 70 per cent has been achieved (9, 21). Yet in the air as little as 0.5 mg. of propylene glycol per liter can almost completely sterilize an atmosphere containing hundreds of thousands of these bacteria per cubic foot (5). Moreover, this action occurs within a space of 15 seconds or less. Triethylene glycol, which is even less active than propylene glycol *in vitro*, is almost one hundred times more potent in the air. Both of these chemicals are viscous, hygroscopic liquids, without any particularly reactive chemical groupings, and possessed of a relatively slight volatility. The mechanism of this potent bactericidal action, therefore, is of interest.

Early investigators working on the problem of chemical disinfection of the air, were led to the belief that the essential mechanism involved consists of collisions between the bacteria-containing droplets and aerosol particles of the germicidal agent (1, 18, 22, 23). This conclusion was based largely on reports that phenolic compounds produced little effect on air-borne bacteria in concentrations below that required to saturate the atmosphere (18, 24, 25). An exception to this viewpoint was that of Masterman (17, 26) who presented evidence to show that the activity of sprays of sodium hypochlorite solution is due to the release of HOCl gas from the atomized droplets by reaction with the CO_2 of the air. Nevertheless, other workers, reinvestigating this phenomenon, concluded that the most potent action of hypochlorites, like that of other aerial germicides is due to the action of the mist particles rather than of the vapor (27).

As a result of these considerations, efforts were expended to produce germicidal aerosols from which evaporation of the active substance could be diminished or entirely suppressed (18, 25, 27) since it was believed that escape of the germicide from such particles by evaporation, rendered it useless for aerial disinfection. The atomization of various mixtures has been recommended and diverse effects dealing with the relationship of bactericidal efficiency to the persistence of such aerosol mists, have been ascribed to the relative humidity and the temperature of the atmosphere, and the vapor pressure and hygroscopicity of the components of the solutions nebulized (12, 18, 25, 28, 29). However, it is not possible to secure from any of these data, a clear explanation for the action of these factors nor to predict accurately how any particular alteration in either the environmental conditions or the composition of the substance employed as the disinfectant, will affect the bactericidal efficiency.

Theoretical Analysis of the Mechanism

The inadequacy of the "germicidal aerosol" concept becomes obvious from a consideration of the velocity of the killing action which is observed. In order to analyze the dynamics of the process, the particle size of the bacterial mists and of the propylene glycol sprays used in our previous experiments (4, 5) was measured by means of a cascade impactor (30).[2] These measurements make

[2] We are indebted to Mr. Lawrence Sonkin for his assistance and for the loan of his modified impactor which was used in these measurements.

it possible to calculate the velocity of the bactericidal action which is to be expected by each of the two mechanisms: *i.e.*, first by assumption that the killing process must be preceded by collisions between glycol aerosol particles and the air-borne bacteria; and second by the assumption that the fundamental process consists of condensation of molecules of glycol vapor on to the bacterial particles leading to accumulation of a lethal concentration of the bactericidal agent about the microorganisms. The detailed calculations are presented in the Appendix. They show that under the experimental conditions here employed collision processes between aerosol particles of the disinfectant and 90 per cent of the air-borne bacteria would require many hours for completion. On the other hand, this same quantity of glycol in vapor form could condense on air-suspended bacterial particles rapidly enough to cause the accumulation of a lethal concentration in each droplet within a matter of seconds. The

TABLE I

A Typical Experiment Showing Killing of a Suspension of Staphylococcus albus Sprayed into Air Containing 0.3 Mg./Liter of Propylene Glycol

Settling plate No.	Time at which plate exposure began	Nos. of microorganisms recovered on each 2 min. settling plate	
		Test chamber	Control number
1	During bacterial spray	21	203
2	1 min. after end of spray	0	311
3	3 " " " " "	0	302
4	5 " " " " "	0	211
5	7 " " " " "	0	212
6	9 " " " " "	0	211
7	11 " " " " "	0	208

enormous rapidity of the vapor condensation process though striking, is readily understandable in view of the tremendously high velocities of free vapor molecules, and the relatively large surface area which the minute bacterial droplets offer. The killing action which was actually obtained in the particular experiment whose velocity has been calculated by these two mechanisms is presented in Table I. The fact that under these conditions, complete sterilization occurs within 1 minute after introduction of the bacteria, clearly rules out the possibility that an aerosol collision mechanism could have been involved.

Experimental Verification of Vapor Mechanism

It is possible to demonstrate the necessity for the existence of the germicidal material in the vapor phase by direct experimental means. First, tests were performed wherein the formation of aerosol droplets was completely excluded. These revealed that pure vapors of glycols and other substances are highly bactericidal, and act extremely rapidly on air-borne bacteria.

(*a*) Four small Petri dishes containing liquid propylene glycol were placed on the floor of a 2-cubic foot chamber (4) which was then sealed. The chamber was contained inside a large, thermostated, constant humidity room, where wet and dry bulb temperatures could be kept constant within 1 to 2°F., for indefinite periods of time (32). The chamber was allowed to remain undisturbed for about 18 hours, at a temperature of 82°F. Thus, the only means for the glycol to enter the air was by evaporation from the exposed liquid surfaces, and the maximum concentration of glycol vapor which could be achieved under these conditions is the saturation value at this temperature. Under these conditions it is thermodynamically impossible for the air inside such a chamber to become supersaturated, so aerosol droplets of glycol cannot form. At the end of the interval allowed for evaporation, a suspension of *Staphylococcus albus* in broth was sprayed into the chamber and 2 liter samples of the air were withdrawn by means of the bubbler sampler (33) at intervals of 15 seconds, 5 minutes, and 15 minutes after the cessation of the bacterial spray.[3] An identical procedure was carried out in a control chamber, except that non-volatile mineral oil instead of propylene glycol was placed in the Petri dishes. Aliquots of the sampling fluid from each chamber were plated and incubated for 48 hours. 15 seconds after the end of the bacterial spray, there was a 73 per cent reduction in the number of bacteria recovered from the air containing propylene glycol vapor as compared with that in the control chamber. At 5 minutes there was a 95 per cent reduction, and at the end of 15 minutes the air containing glycol vapor was sterile while air from the control chamber still contained over a thousand bacteria per liter.

Completely analogous results have been obtained when various other substances were used instead of propylene glycol. Thus, saturated atmospheres of ethyl alcohol, ethylene glycol, tetrahydro furfuryl alcohol, and 2-amino 2 ethyl 1-propanol were found to be highly lethal toward air-borne bacteria. It was difficult to establish by this means, the vapor action of triethylene glycol because of its extremely slow rate of evaporation. Hence, for this compound, the vapor effect was demonstrated by allowing evaporation to occur from a large sheet which was moistened with liquid glycol, and then hung up overnight in the large constant temperature room. The next day, the sheet was quickly removed from the room with a minimum disturbance of its atmosphere. A standard suspension of *Staphylococcus albus* was sprayed into the room and the air was sampled at periodic intervals. A control experiment was run in a duplicate chamber. It was found that the presence of glycol vapor evaporated into the air from this sheet produced killing of 90 per cent of the air-borne staphylococci within 15 minutes and almost complete sterilization of the air at the end of 25 minutes. During the same period the number of bacteria in the control experiment remained practically constant.

(*b*) Bactericidal action in the absence of any demonstrable aerosol particles of germicide was shown in yet another way.

An intense collimated beam of light was produced by means of a 6 volt auto headlight lamp, placed at the focus of a lens 5.8 cm. in diameter and 14 cm. in focal length. The beam was directed across the experimental chamber so as to reveal the presence of even a highly

[3] The validity of the sampling methods employed, and the demonstration that the effects observed produce true death of the microorganisms, and not simply bacteriostasis have been described in earlier publications (1, 7).

dilute aerosol by means of the Tyndall effect. Propylene glycol was vaporized into the chamber, but in an amount insufficient to produce any visible Tyndall beam whatever. A bacterial suspension of *Staphylococcus albus* was then sprayed into the chamber, and bacterial samples of the air were taken.[4] Within 2 minutes after the end of the bacterial spray, the atmosphere was completely sterile. In a control experiment where the procedure was duplicated in the absence of the glycol the air was found to contain several thousand bacteria per cubic foot.

The foregoing experiments demonstrate fairly conclusively that pure vapors, in the absence of any aerosols can be highly bactericidal. The converse experiments have also been performed; *i.e.*, aerosols in which all of the germicide has been confined within particulate droplets and none whatever allowed to exist in the vapor phase have been found to have no effect whatever on air-borne microorganisms, within a period of at least 20 minutes.

For these experiments aerosols of zephiran[5] were employed. This substance is highly lethal to β hemolytic streptococci, even in dilutions of 1:50,000 or more (34). Its chemical structure is that of a quaternary ammonium salt, so that it possesses practically no volatility whatever. 0.5 gm. of a 10 per cent aqueous solution of zephiran was sprayed into the 640 cubic foot experimental room, which was kept at a temperature of 73°F. and 50 per cent relative humidity. The atomizer selected for this spray produces a very fine particle size whose mass median radius was found to be 0.95 μ.[6] This size lies within the range of 0.5 to 1.0 μ which has been described as the most effective particle size range for germicidal aerosols (18). A dense fog of the zephiran aerosol filled the air of the chamber. Yet despite the tremendous germicidal potency of the zephiran, and the high concentration of its aerosol droplets, no killing action whatever could be demonstrated on beta hemolytic streptococci sprayed into the air of the chamber. In a series of ten air samples taken over a period of 20 minutes the numbers of these microorganisms recovered from the air of the test chamber were almost identical with those obtained from the control chamber. This type of experiment would seem to be irreconcilable with the theory that a germicidal aerosol is the active agent involved in aerial disinfection.

DISCUSSION

It must be concluded, therefore, on the basis of both theoretical and experimental grounds that rapid killing of air-borne bacteria in concentrations of the order of magnitude of those described here, requires the existence of the germicide in the vapor phase. This concept has proved to be a simplifying and useful one. It led directly to the demonstration of triethylene glycol and other compounds as aerial disinfectants tremendously more potent than propylene glycol (6, 35). It explained the nature of the influences exerted on this bactericidal process by a number of factors which will be described in the succeeding paper of this series (13). On the basis of this principle it has been possible to design an instrument which, by regulating

[4] As soon as the bacterial spray was introduced, a very heavy Tyndall beam appeared.

[5] Alkyl dimethyl benzyl ammonium chloride.

[6] *I.e.* this means that 50 per cent of the mass of this aerosol was distributed in particles whose radius was less than 0.95 μ.

the concentration of the germicidal vapor in the air, permits accurate control of the lethal process to be achieved (40, 81).

The experimental results reported here, indicate the tremendous rapidity with which an aerosol can interact with a soluble vapor, even when the latter is present in very low concentrations. The equation governing the rate of condensation of vapor molecules on to a spherical droplet (Equation 6, Appendix) assumes that every collision is effective. The fact that propylene glycol whose vapor pressure at 25°C. is 0.13 mm. Hg (39), reacts with an aerial dispersion of microorganisms within a time comparable to that demanded by this equation, indicates that a high degree of efficiency is obtained for this process. Even triethylene glycol, with a vapor pressure of 0.0013 mm. Hg (39) produces extensive killing of air-borne microorganisms within a few minutes, when present in concentrations well below the saturation point.

APPENDIX

Calculation of Rate of Interaction of a Germicide with Air-Suspended Bacteria

In a typical experiment, 6.20 gm. of propylene glycol was sprayed from a calibrated atomizer, into a 640 cubic foot chamber, at a temperature of 73°F. and a relative humidity of 65 per cent. Then 0.28 gm. of a standard suspension of *Staphylococcus albus* culture was sprayed into the same chamber by means of a second calibrated atomizer. Measurement with a cascade impactor (30) revealed that the bacterial droplets produced inside the chamber under these conditions, have a mass median radius r, equal to 0.32×10^{-4} cm.[7] Thus, the mass of the average bacterial droplet, $4/3\pi r^3 d$, (where r = radius of the particle, and d is its density, taken as one) is 1.37×10^{-13} gm. and the number of bacterial droplets per cc., n_b is equal to

$$\frac{0.28}{1.37 \times 10^{-13} \times 640 \times 28{,}300} = 1.13 \times 10^{6}$$

A. Rate of Kill Expected by the "Germicidal Aerosol" theory.—For this calculation we shall assume that the propylene glycol droplets introduced into the chamber, do not evaporate further so that the maximum quantity of "germicidal aerosol" is available for bactericidal action. The mass median radius of propylene glycol droplets immediately emergent from the atomizer was found by measurement with a cascade impactor, to be 1.1×10^{-4} cm. Hence, the mass of the average particle entering the chamber is $4/3\ \pi r^3\ (d) = 5.8 \times 10^{-12}$ gm. Thus n_g, the number of such "germicidal aerosol" particles per cc. of air in the chamber is

$$n_g = \frac{6.20}{5.8 \times 10^{-12} \times 640 \times 28{,}300} = 5.9 \times 10^{4} \text{ particles per cm.}^3$$

The collision frequency between aerosol particles of one kind with those of another is given by Smoluchowski's equation (31) corrected to account for inhomogeneities in the gaseous medium:

$$\frac{-dn_b}{dt} = \frac{RT}{3_\eta N} \frac{(r_b + r_g)^2}{r_b r_g} n_b n_g \left(1 + \frac{AL}{\bar{r}}\right) \qquad (1)$$

[7] For the purpose of the present calculations, it is sufficiently accurate to treat these clouds as consisting of uniform particles of this radius (36).

where R = gas law constant, T = absolute temperature, η = viscosity of air; N = Avogadro's number; r_g and r_b are the radii of the germicide droplets and bacterial droplets respectively; n_b and n_g are the respective numbers of each droplet species present in 1 cc. of air; AL is the Cunningham correction term (31) and $\bar{r}$ is a mean value of r_b and r_g. On theoretical grounds this equation has been shown to be valid within a few per cent for any particle size greater than 10^{-5} cm. in radius (37). It has also been verified experimentally over a wide range of particle sizes by Whytlaw-Gray and his coworkers (31). The only assumption involved in our use of this equation is the value of r_g, the radius of the germicide aerosol, which we shall take as the measured value of 1.1×10^{-4} cm., neglecting any effect of evaporation. This assumption, however, can only lead to a collision frequency greater than the actual one, because as r_g decreases, the collision velocity also decreases, reaching a minimum at $r_g = r_b$. Further diminution in r_g again raises the collision velocity but since a concentration in solution of at least 50 per cent propylene glycol is required to produce death of these microorganisms within 30 minutes or less (21) any droplet smaller than r_b cannot contribute appreciably to any rapid killing process.

We shall calculate the time which would be required for killing 90 per cent of the bacteria by this collision process. We may write:

$$\frac{dn_b}{dt} = kn_b n_g \tag{2}$$

where

$$k = \frac{-RT}{3\eta N} \frac{(r_b + r_g)^2}{r_b r_g} \left(1 + \frac{AL}{\bar{r}}\right) \tag{3}$$

$R = 8.31 \times 10^7$ dynes cm./mole deg.; $T = 296°$A.; $r_b = 0.32 \times 10^{-4}$ cm.; $r_g = 1.1 \times 10^{-4}$ cm.; $\eta = 1.83 \times 10^{-4}$ poises; $AL = 9 \times 10^{-6}$ cm. for air at room temperature and $\bar{r} = \frac{1}{2} 10^{-4}$ cm. Hence

$$k = -5.0 \times 10^{-10} \frac{\text{cm.}^3}{\text{sec.}}$$

For simplicity let us further assume that n_g, the particles of germicide available for collision, does not diminish with time, but remains at its original value, of 5.58×10^4 throughout. This assumption again will only increase the apparent killing velocity.

Then:

$$\frac{dn_b}{n_b} = -(4.65 \times 10^{-10} \times 5.9 \times 10^4)\, dt \tag{4}$$

$$2.303 \log \frac{n_b}{n_b^o} = -2.8 \times 10^{-5} \Delta t$$

For 90 per cent killing,

$$\text{Log} \frac{n_b}{n_b^o} = \log 0.1 = -1$$

therefore,

$$\begin{aligned} \Delta t &= \frac{(2.303)(-1)}{-2.6 \times 10^{-5}} \\ &= 8.2 \times 10^4 \text{ seconds} \\ &= 22.8 \text{ hours} \end{aligned} \tag{5}$$

Twort and his associates (18) realized that an aerosol collision mechanism would require much more time for completion than if the germicide operated *via* the vapor state. However, they were unable to reconcile this calculation with their apparent failure to observe killing action unless supersaturated atmospheres of disinfectant were employed. Hence, they concluded that "vapours of the germicide are useless" (Twort (18), p. 339) and attempted to ascribe the discrepancy either to error in the Smoluchowski formula (Equation 1) or to the existence of an extremely small, and highly active aerosol particle of germicide which would be the functional agent of aerial disinfection. The first of these possibilities is ruled out by the several independent verifications of the Smoluchowski equation (31, 37) and no experimental evidence supporting the second has ever been put forward. Moreoever the demonstration that propylene glycol can kill air-borne bacteria even more quickly than *n*-hexyl resorcinol disproves this latter hypothesis, since a bacterial particle would have to collide with a glycol droplet at least as large as itself, if a single collision were to produce a lethal concentration of germicide about the microorganism. An explanation for the observations of Twort and his associates is suggested in the second paper of this series (13).

B. Rate of Kill Expected by the Vapor Condensation Mechanism.—This calculation resolves itself into computing the rate of condensation of sufficient glycol vapor molecules to produce a rapidly lethal concentration of glycol on the bacteria-containing droplets. For the present purpose, it will be sufficient simply to demonstrate the time order of magnitude necessary for such a process. Condensation of vapors on a liquid droplet proceeds at a velocity such that the rate of increase of area with time, is constant (38).

$$\frac{dA}{dt} = \frac{8\pi DM}{RTd}(p_g - p_g') \tag{6}$$

where A = area of droplet,
D = diffusion coefficient of the vapor,
M = molecular weight of the vapor,
R = gas law constant,
T = absolute temperature,
d = density of liquified vapor,
p_g = partial pressure of the vapor in the air,
p_g' = pressure of the vapor at the surface of the droplet.

In this calculation, we shall consider the effect of the propylene glycol vapor which results when a spray is introduced into the chamber. Under the conditions of the experiment (*i.e.* relative humidity of 65 per cent, excess propylene glycol present) evaporation would proceed until a vapor concentration of (1–0.65) p_g^o was attained ((13), Equation 6) which in this case would be 0.034 mm. Hg at 73°F. (39). We shall substitute this value of p_g in equation (6). The value of p_g', the partial pressure of glycol at the surface of the absorbing droplet, is zero at the moment of introduction of the bacterial spray. As condensation proceeds, this value rises, ultimately reaching a level equal to that in equilibrium with the surrounding atmosphere, 0.034 mm. Hg. We are interested in the time necessary to deposit an almost instantly lethal concentration in the droplet, which may be set at 65 per cent by weight (14). This corresponds to a mole fraction of $\frac{\frac{65}{76}}{\frac{65}{76}+\frac{35}{18}}$ = 30.6 per cent, and the partial pressure of propylene glycol above such a solution is 0.306 p^o = 0.030 mm. Hg. We shall resort to a simplification, substituting this value for p_g' in equation (6). The effect of this approximation will be to increase the apparent time necessary for achievement of the lethal dose.

Molecules of glycol and water vapors are now both condensing on the particle in a ratio of $\frac{0.35}{0.65}$. Just how much total volume of a solution of this strength is required to kill a bacterium, is as yet unknown. However, it seems reasonable to assume that a quantity of this lethal solution containing twenty times as much glycol as the weight of the original bacterial particle, would be sufficient to bring about death of the microorganism. In this calculation, we may disregard completely the condensation of the water vapor, because its much greater vapor pressure and diffusivity make the time necessary for its condensation negligibly small compared to that of the glycol. Hence, it becomes necessary to calculate only the time necessary for condensation on the bacterial particle of an amount of glycol equal to $20 \times 1.37 \times 10^{-13}$ gm. $= 2.74 \times 10^{-12}$ gm. This quantity of glycol condensed by itself on the original bacterial particle would produce a new droplet whose mass is $(2.74 + 0.14) \times 10^{-12} = 2.88 \times 10^{-12}$ gm. and whose area is 9.8×10^{-8} cm.^{2}. Hence, we may use for dA, the value $\Delta A = (9.8 \times 10^{-8}$ cm.$^2) - (4\pi)(0.32 \times 10^{-4})^2 = 8.5 \times 10^{-8}$ cm.^{2}. We can now solve for Δt, the time interval necessary for the condensation of an amount of glycol which would be instantly lethal to the average airborne bacterial particle with which we are dealing:

$$\Delta t = \frac{RTd}{8\pi DM} \frac{\Delta A}{(p_g - p_g')} \tag{7}$$

R, the gas constant, is $62,400 \frac{\text{cc.} \times \text{mm. Hg}}{\text{mole deg.}}$; $T = 296°$A; d, the density of liquid propylene glycol = 1.04; $\Delta A = 8.5 \times 10^{-8}$ cm.^{2}; $M = 76$ gm./mole; D, the diffusion constant for propylene glycol vapor in air is not known, but by analogy with molecules of similar structure (31) cannot be less than 0.05 cm.^{2}/second; and $p_g - p_g' = 0.034 - 0.030 = 0.004$ mm. Hg. By substitution, then

$$\Delta t = 4 \text{ seconds} \tag{8}$$

To this value of 4 seconds it would be necessary to add about 15 seconds for the interval required to effect uniform mixing of the air in the experimental chamber, giving a total of about 20 seconds for the order of magnitude of the expected killing time.

SUMMARY

Theoretical analysis of the mechanism of action of chemical aerial disinfectants reveals that the rapid killing action which is obtained cannot be accounted for by a collision process between germicidal aerosol particles and the air-borne bacteria. However, a mechanism involving condensation of germicide molecules in the vapor state on to the bacteria-containing droplets results in a theoretical velocity of the correct order of magnitude.

Experimental tests of this theory show that pure germicide vapors free of aerosol droplets are almost instantly lethal to air-borne bacteria. Conversely, pure germicidal aerosols in the absence of vapor, had no effect on air-borne bacteria within 20 minutes or more. Therefore, it may be concluded on both theoretical and experimental grounds that rapid air sterilization requires the existence of the germicide in the vapor state.

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BIBLIOGRAPHY

1. Robertson, O. H., Bigg, E., Miller, B. F., and Baker, Z., *Science*, 1941, **93**, 213.
2. Robertson, O. H., Bigg, E., Miller, B. F., Baker, Z., and Puck, T. T., *Tr. Assn. Am. Physn.*, 1941, **56**, 353.
3. Robertson, O. H., Bigg, E., Puck, T. T., Miller, B. F., and Baker, Z., *Am. Assn. Advance. Sc.*, Pub. No. 17, 1941, 271.
4. Robertson, O. H., Bigg, E., Puck, T. T., and Miller, B. F., *J. Exp. Med.*, 1942, **75**, 593.
5. Puck, T. T., Robertson, O. H., and Lemon, H. M., *J. Exp. Med.*, 1942, **78**, 387.
6. Robertson, O. H., Puck, T. T., Lemon, H. M., and Loosli, C. G., *Science*, 1943, **97**, 142.
7. Hamburger, M., Puck, T. T., and Robertson, O. H., *J. Infect. Dis.*, 1945, **75**, 208.
8. Puck, T. T., Hamburger, M., Robertson, O. H., and Hurst, V., *J. Infect. Dis.*, 1945, **76**, 216.
9. Robertson, O. H., *Harvey Lectures*, 1942, **38**, 227.
10. DeOme, K. B., and Personnel of Naval Laboratory Research Unit No. 1, *Am. J. Hyg.*, 1944, **40**, 239.
11. Personnel of Naval Laboratory Research Unit No. 1, *U. S. Nav. Med. Bull.*, 1944, **42**, 1288.
12. Baker, A. H., and Twort, C. C., *J. Hyg.*, Cambridge, Eng., 1944, **43**, 382.
13. Puck, T. T., *J. Exp. Med.*, 1947, **85**, 741.
14. Puck, T. T., and Dunklin, E., data to be published.
15. Lister, J., *Brit. Med. J.*, 1871, **2**, 225.
16. Douglas, S. R., Hill, L., and Smith, W., *J. Ind. Hyg. and Toxicol.*, 1928, **10**, 219.
17. Masterman, A. T., *J. Ind. Hyg. and Toxicol.*, 1938, **20**, 278.
18. Twort, C. C., Baker, A. H., Finn, S. R., and Powell, E. O., *J. Hyg.*, Cambridge, Eng., 1940, **40**, 253.
19. Robertson, O. H., *Science*, 1943, **97**, 495.
20. Personnel of Naval Laboratory Research Unit No. 1, *War Medicine*, 1943, **4**, 1.
21. Robertson, O. H., Appel, E., Lemon, H. M., Puck, T. T., and Ritter, M., data to be published.
22. Pulvertaft, R. J. V., and Walker, J. W., *J. Hyg.*, Cambridge, Eng., 1939, **39**, 696.
23. Trillat, A., *Bull. Acad. med.*, 1938, **119**, series 3, 64.
24. Bechhold, H., British Patent 1935, 472623. Cited by Twort *et al.* (18).
25. Finn, S. R., and Powell, E. O., *Nature*, 1939, **144**, 864.
26. Masterman, A. T., *J. Hyg.*, Cambridge, Eng., 1941, **41**, 44.
27. Baker, A. H., Finn, S. R., and Twort, C. C., *J. Hyg.*, Cambridge, Eng., 1940, **40**, 560.
28. Williamson, A. E., and Gotaas, H. B., *Ind. Med.*, 1942, **11**, *Ind. Hyg. Sect. 3*, 40.
29. Ornstein, O., and Thorsson, K. G., *Acta Path. Microbiol. Scand.*, 1944, **21**, 914.
30. Sonkin, L., *J. Ind. Hyg. and Toxicol.*, 1946, **28**, 269.
31. Whytlaw-Gray, R., *Tr. Faraday Soc.*, 1936, **32**, 1042. Whytlaw-Gray, R., and Patterson, H. S., Smoke, London, Edward Arnold and Co., 1932.
32. Robertson, O. H., Puck, T. T., and Wise, H., *J. Exp. Med.*, 1946, **84**, 559.
33. Lemon, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1943, **54**, 298.
34. Miller, B. F., and Baker, Z., *Science*, 1940, **91**, 624.

35. Lovelock, J. E., Lidwell, O. M., and Raymond, W. F., *Nature*, 1944, **153**, 20.
36. Cawood, W., and Whytlaw-Gray, R., *Tr. Faraday Soc.*, 1936, **32**, 1063.
37. Fuchs, N., *Tr. Faraday Soc.*, 1936, **32**, 1147; *Z. physik. Chem.*, 1934, **171**, 199.
38. Langmuir, I., *Physic. Rev.*, 1918, **12**, series 2, 368; and Houghton, H. G., *Physics*, 1933, **4**, 419.
39. Puck, T. T., and Wise, H., *J. Physic. Chem.*, 1946, **50**, 329.
40. Puck, T. T., Wise, H., and Robertson, O. H., *J. Exp. Med.*, 1944, **80**, 377.