DISINFECTION OF CLOUDS OF MENINGOPNEUMONITIS AND PSITTACOSIS VIRUSES WITH TRIETHYLENE GLYCOL VAPOR*

By THEODOR ROSEBURY, D.D.S.,

GORDON MEIKLEJOHN, M.D.,

Lieutenant, Medical Corps, United States Naval Reserve,

LAWRENCE C. KINGSLAND, M.D.,

Lieutenant, Medical Corps, United States Naval Reserve,

AND MARTIN H. BOLDT, M.D.,

Captain, Medical Corps, Army of the United States

(From the Laboratories of Camp Detrick, Maryland)

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Studies at Camp Detrick, and many previous studies elsewhere, have indicated that accidental infections occurring in laboratories in which highly infective bacterial or viral agents are studied may be due to inhalation of an aerosol of the agent. Although some progress has been made toward elimination of laboratory infections at the source (1–3) this approach has not always been successful. A study of measures for specific disinfection of air was therefore considered desirable.

Among the methods available for disinfection of air, the use of germicidal aerosols appeared best suited to the present purpose in view of their capacity for uniform dissemination, their low toxicity, and evidence that their activity may be limited to air-borne particles. Of the many potential disinfectants tested by French, British, and American workers, triethylene glycol was selected because of its apparent effectiveness in extremely low vapor concentrations.

The earlier studies on air disinfection, based on present day theory of air-borne infection, have been ably reviewed up to 1941 by Buchbinder (4). In the earliest studies, substances were selected for test as air disinfectants on the basis of their known germicidal activity in solution; *e.g.*, sodium hypochlorite and hexylresorcinol. It then appeared, however, that the activity of germicides in air was not necessarily correlated with their activity in solution (5). Twort, Baker, Finn, and Powell (5) had used propylene glycol as a vehicle for hexylresorcinol. Shortly thereafter, Robertson, Bigg, Miller, and Baker (6) reported that propylene glycol alone was very effective when dispersed

* Studies conducted at Camp Detrick, Maryland, from December, 1944, to July, 1945.

from a nebulizer, both in destroying the viability of static clouds of several common bacterial species, and in abolishing the infectivity for mice of clouds of type I pneumococci. Robertson and his associates (7) later found that propylene glycol dispersed as vapor rather than as an aerosol killed *Streptococcus hemolyticus* in a static cloud within 15 minutes at a concentration in air of 1:7,700,000, and prevented infection of mice with pneumoccocci at a concentration of 1:3,000,000. On the other hand the pneumococcus was found to grow in broth containing as much as 5 per cent of propylene glycol; other organisms grew in the presence of 15 per cent, and the lethal concentration of this substance in solution was estimated as 70 to 80 per cent.[1] It was suggested that a lethal concentration develops at very low vapor concentrations by condensation of glycol vapor in suspended bacteria-laden droplets.

Further details and an amplification of this view were given in a later report by Puck, Robertson, and Lemon (8). Propylene glycol vapor, when introduced at levels in excess of vapor saturation, was found to be very rapidly bactericidal for static clouds of all of 12 common varieties of parasitic and pathogenic bacteria, but wide variation in killing rate was reported with vapor levels below saturation. The pneumococcus was found to be most sensitive and *Staphylococcus albus* more resistant. The germicidal activity of propylene glycol vapor was found to increase as the temperature in the experimental chamber was lowered from 37 to 15°C., and to vary with relative humidity, showing maximum activity between 50 and 65 per cent and falling off rapidly on both sides. The temperature effect was interpreted in terms of decreasing vapor pressure of glycol with decreasing temperature, favoring condensation on suspended particles. It was further suggested that the glycol condenses in the aqueous phase of suspended particles, so that it is ineffective at low relative humidities; while at high humidities the glycol is distributed among empty as well as bacteria-laden water droplets, and thus fails to reach optimum concentration in the latter.

These workers also reported that propylene glycol vapor, in a concentration of 1:2,000,000, prevented infection of mice exposed to a static cloud of the PR8 strain of influenza virus. The cloud concentration was otherwise sufficient to kill all of 35 mice within 10 days (9). In a more recent report (10) similar data were given for triethylene glycol, which was found active against static clouds of influenza virus and also of pneumococci and hemolytic streptococci. Triethylene glycol was said to compare favorably with propylene glycol with respect to low toxicity, and to be superior to the latter and to other glycols tested in that much lower vapor concentrations were active.

[1] In unpublished studies by two of the writers, using the method kindly suggested by Major C. G. Loosli, Army of the United States, triethylene glycol has been found to kill psittacosis virus (strain 6 BC) in broth at a glycol concentration of 80 per cent but not at 60 per cent, as tested by inoculation both of mice and of chick embryos.

Working with a dynamic cloud of *Salmonella pullorum* and with triethylene glycol, DeOme and his collaborators (11) reported significant killing effects of vapor in low concentrations (1:100,000,000 or lower) at relative humidities between 42 and 62 per cent, but not at 15 per cent or at 79 per cent. The data suggest that within the effective range of humidity, killing is somewhat more marked at lower humidities; and the authors selected 45 per cent relative humidity as the optimum for germicidal activity. The killing effect was found to be greater at 28°C. than at 36°C. With propylene glycol at 36°C. and 45 per cent relative humidity, a well marked progressive germicidal effect occurred as the concentration of vapor in the cloud increased from 1:5,000,000 to 1:690,000. Complete killing within 15 seconds was observed only at the highest of these levels.

The apparently narrow range of relative humidity required for glycol activity has been emphasized by Robertson and his collaborators (12), and has been advanced by British authors (13) as the reason for their failure to recommend triethylene glycol for general use as an air disinfectant.

Methods

Cloud Chamber Apparatus.—Reference must be made to another report (3) for details of the cloud chamber methods used, which are essentially new and can be only briefly recapitulated within the scope of the present paper. The cloud chamber employed was part of a completely closed system which included devices for animals that allowed them to be introduced, removed, maintained for study, autopsied, examined, and disposed of, all without leakage of the infective agent into the working environment. The atomizing system and the cloud-sampling devices were placed and operated within the chamber. Two types of atomizer system were used for producing infective clouds. Both were direct spray atomizers, like miniature paint sprayers, which operate without refluxing or concentrating the suspension being sprayed. One of these (the "Chicago" atomizer), an all glass instrument adopted and used routinely in all cloud chamber experiments after initial comparative studies, has been found to produce clouds with a particle size distribution only slightly coarser than that of nebulizers (refluxing internal spray atomizers; *e.g.*, the De Vilbiss 40 or 180), which are generally more destructive to infective agents. The atomizer was assembled with a cylindrical glass "mixing chamber" to which a compressed air line separate from that which operates the atomizer was connected. This "secondary" air dilutes and dries the spray, and serves to control both the total air flow through the chamber and the relative humidity of its atmosphere. Fluid capillary impinger samplers were used for sampling the cloud. These impingers consist of a collecting tube ending in a capillary which dips into suitable collecting fluid in a 125 ml. filtering flask. The capillaries act as critical orifices, so that they may be calibrated individually for rate of air flow and used without flowmeters. The flow of air through all entering lines was measured with appropriate flowmeters.

Vaporization of Triethylene Glycol.—Triethylene glycol vapor[2] was generated in the first two series of experiments with an experimental room glycol vaporizer.[3] The vapor port of

[2] The triethylene glycol used in these studies was "Air Sterilization Grade," obtained from Union Carbon and Carbide Company, New York.

[3] We are grateful to the Research Corporation, Bound Brook, New Jersey, for the use of this instrument.

this instrument was closed with heavy rubber dam, to which a 3 foot length of rubber pressure tubing, ¼ inch in bore, was attached with the aid of a perforated stopper wedged between the sides of the port. The tube was led directly to an opening in the cloud chamber. The very low static pressure of this vaporizer made it necessary to calibrate it for rate of flow of vapor by drawing the vapor through a standard orifice under vacuum, with a water manometer at the upstream end of the standard orifice tube, corresponding to the point of entrance of the glycol vapor into the cloud chamber. The delivery of vapor against a vacuum of ½ inch of water (the pressure generally maintained in the cloud chamber) was adjusted under these conditions to 27 to 30 liters per minute. This vapor flow was found to be sufficient to produce a light but appreciable fog in the chamber, indicating the presence of glycol in excess of saturation.

In the third and subsequent series of experiments, glycol vapor was produced with the aid of a De Vilbiss 180 nebulizer placed in a boiling water bath. The air operating this nebulizer was preheated by passing it through a coil of ¼ inch outside diameter copper tubing in the water bath before it was run into the atomizer. A flowmeter, in an air line that was used only for the glycol vaporizer, was calibrated with this instrument *in situ*, and operated in all experiments at a flow rate of 6 liters per minute. With a total air flow through the chamber of about 90 liters per minute, the rate of glycol flow (0.14 to 0.15 ml. per minute) was found to yield a sufficient excess over saturation so that a distinct fog became visible in the chamber in less than 5 minutes. When the glycol flow was discontinued and replaced by air run at the same rate through the empty De Vilbiss atomizer, with other conditions held constant, the glycol fog disappeared in an approximately equal period.

Virus Preparations.—Suspensions of meningopneumonitis virus (strain Cal 10[4]) and of psittacosis virus (strain 6 BC[4]) were prepared from yolk sacs harvested from chick embryos which had died 6 to 8 days after yolk sac inoculation. The pooled sacs were weighed, ground with glass beads, diluted 1:10 in extract broth, distributed into ampules, and preserved by freezing in CO_2 ice. For use the contents of an ampule were further diluted in extract broth to the concentration desired. The titers of the yolk sacs before dilution, by intracerebral inoculation of mice (0.03 ml.) were, for Cal 10, approximately 10^{-8}, and for 6 BC, approximately 10^{-9}.

Both viruses were sprayed from suspension in extract broth except in the first series of experiments, in which chick allantoic fluid was used as diluting medium. Cloud samples were collected in capillary impingers with rates of flow between 2 and 3 liters per minute. The samplers were operated after the cloud had attained approximate equilibrium concentration. The collecting medium used was extract broth, 25 ml. in amount, to which 4 drops of olive oil were added to limit foaming. Two impingers were used for each sampling. Virus concentration was estimated both in suspensions sprayed and in impinger fluid by intracerebral titration in mice, using 8 mice per dilution with at least four dilutions at tenfold intervals. LD_{50} values were calculated from the mortality data in these mice by the method of Reed and Muench (14). These mouse intracerebral LD_{50} values are employed as infective units of virus, equivalent for purposes of calculation to "organisms" as applied to a cultivable bacterial agent.

Calculations.—Values given in the tables for "nominal recovery" were derived as the average concentration of virus (in intracerebral LD_{50} units) per liter of cloud recovered, expressed as a percentage of the concentration of virus sprayed. The concentration recovered was obtained from the impinger titration data (LD_{50} per ml.) multiplied by the volume of impinger fluid (25 ml.) and divided by the air flow in liters through the samplers during the sampling interval. The concentration sprayed was determined from the suspension titration data multiplied by the atomizer fluid flow (by weight) and divided by the total air flow

[4]Strains Cal 10 and 6 BC were obtained, respectively, through the courtesy of Dr. M. D. Eaton and Dr. K. F. Meyer.

through the chamber during the spraying period. Data given elsewhere (3) show that this procedure, based on samples taken during the period of equilibrium concentration of cloud, gives a valid measure of the true recovery.

Mouse dosage was estimated in terms of intracerebral LD_{50} units. The dose was calculated from the values for concentration recovered, by multiplying these values, in terms of intracerebral LD_{50} units per ml. of cloud, by the average respiratory volume of the mouse (1.25 ml. per minute per gm. body weight) (15) and by the average weight in grams of the animals.

Estimation of Infective Response.—Mice exposed to meningopneumonitis virus were sacrificed in groups from the 4th to the 11th day after exposure. Degree of infective response was estimated in relative terms by counting the number of typical focal lesions on all lung surfaces. The first group of mice that were exposed to psittacosis virus (series IV) were killed on the 9th day after exposure, while those of series V were killed on the 7th day. In addition to discrete pulmonary lesions, massive pulmonary consolidation was observed in some animals. At the respiratory dosage levels used few mice died within 9 days after exposure, but other experiences in these laboratories with strain 6 BC (3) have shown that mice with massive consolidation would generally die over an experimental period as long as 30 days. On the other hand, animals with discrete lesions frequently proceeded to complete recovery during this long interval. A more sensitive index of infective response was thus obtained in the shorter periods. In the tabulated data for psittacosis virus (Table II) the number of actual deaths has been increased by addition of mice that showed massive consolidation.

EXPERIMENTAL

Protocols

The five series of experiments described represent progressive attempts to solve certain problems of method that presented themselves during the course of the work, and only the fifth series is considered adequately free from defects. These problems were concerned with the occurrence in the experiments of variables other than the presence of glycol vapor. They appear to have stemmed from two technical difficulties: a progressive diminution in the rate of fluid flow through the virus atomizer from run to run in a given series, and the presumably incomplete removal of glycol from the chamber between runs. The first difficulty resulted in a diminished concentration of cloud sprayed in successive runs in a series, while the second may have been responsible for lower yields in control runs that followed glycol runs. Decreasing atomizer fluid output seemed attributable to the use of relatively concentrated virus suspensions, in which particles of yolk sac seemed to cause progressive clogging of the atomizer. The effect was overcome by the use of higher dilutions in broth, and by washing the atomizer between runs by spraying distilled water. The second difficulty was partially eliminated by washing the chamber with clean air for 15 minutes between runs.

For additional data on the following experiments, see Table I.

Series I.—Meningopneumonitis virus. Runs A4.1[5] (control) and A4.2 (glycol). December 13, 1944.

Suspension: Strain Cal 10 (21F3Y)[6]; yolk sacs 1:10 in extract broth, further dilution 1:9 in fresh chick allantoic fluid from Cal 10-infected eggs.

Equipment: Triple-jet atomizer with 2½ inch mixing chamber; room glycol vaporizer.

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[5] Run serial numbers have the following significance: The initial letter (A or B) refers to the use of one of the two essentially identical cloud chambers that were available; the integral number indicates a series of consecutive runs done in sequence on 1 day; the decimal indicates the individual run in this series. The letter "G" indicates that glycol was used.

[6] Signifying 21 fluid and 3 yolk passages.

Schedule: Cloud samples taken and animals exposed simultaneously for 7 minutes, starting 12 minutes after turning on the atomizer. Glycol flow started with the atomizer in run A4.2.

Series II.—Meningopneumonitis virus. Runs A6.1 (blank control), A6.2 (air flow control), A6.3 (glycol). January 9, 1945.

Suspension: Strain Cal 10 (21 F4Y); yolk sacs 1:100 in extract broth, further dilution 1:10 in extract broth.

TABLE I

Experiments with Meningopneumonitis and Psittacosis Viruses

Series	Run No.	Atomizer fluid flow	Air flow				Dry bulb temperature	Relative humidity	Concentration sprayed	Cloud concentration	
			Atomizer	Mixer	Glycol atomizer	Total				Sprayed	Recovered
		gm./min.	*liters per min.*				°C.	*per cent*	LD_{50}/*0.03 ml.*	LD_{50}/*liter*	LD_{50}/*liter*
I*	A4.1	0.434‡	13.8	60	27	100.8	—	50–60§	1×10^6	1.3×10^5	2.2×10^4
	A4.2G		13.8	60	27(G)	100.8	—	50–60§	1×10^6	1.3×10^5	3.6×10^3
II*	A6.1	0.467	13.8	60	—	73.8	—	35–45§	6.3×10^4	1.3×10^4	1.4×10^3
	A6.2	0.406	13.8	60	30	103.8	—	35–45§	6.3×10^4	8.2×10^3	7.8×10^2
	A6.3G	0.388	13.8	60	30(G)	103.8	—	35–45§	6.3×10^4	7.8×10^3	7.5×10^2
III*	B9.1	0.457	5.5	60	6	71.5	28.5	49	‖	‖	‖
	B9.2G	0.326	5.5	60	6(G)	71.5	28	44			
	B9.3	0.175	5.5	60	6	71.5	28	40			
IV¶	B14.1	0.789	4.8	85	6	95.8	26	50	1.2×10^{5}§	3.3×10^4§	9.5×10^3
	B14.2G	0.784	4.8	85	6(G)	95.8	29	37			6.9×10^2
	B14.3	0.786	4.8	85	6	95.8	28.3	42			2.4×10^3
V¶	B20.1	0.933	4.5	85.5	6	96	27.5	50	1.5×10^6	4.9×10^5	3.2×10^4
	B20.2G	0.886	4.5	85.5	6(G)	96	29	43	1.5×10^6	4.7×10^5	1.1×10^4
	B20.3	0.885	4.5	85.5	6	96	29	43	1.5×10^6	4.7×10^5	3.5×10^4
	B20.4G	0.908	4.5	85.5	6(G)	96	29	42	1.5×10^6	4.8×10^5	1.1×10^4
	B20.5	0.891	4.5	85.5	6	96	29	41	1.5×10^6	4.7×10^5	1.9×10^4

G = triethylene glycol.

* Meningopneumonitis virus (Cal 10).

‡ Measured before run A4.1 and after A4.2.

§ Estimated.

‖ Not determined.

¶ Psittacosis virus (6 BC).

Equipment: Same as series I.

Schedule: Cloud samples taken and animals exposed simultaneously for 10 minutes, starting 10 minutes after turning on the atomizer. Glycol started 10 minutes before starting atomizer in run A6.3.

Series III.—Meningopneumonitis virus. Runs B9.1 (base control), B9.2 (glycol), B9.3 (second control). April 23, 1945.

Suspension: Strain Cal 10 (21F5Y); yolk sacs 1:10 in extract broth, further dilution 1:20 in extract broth.

Equipment: "Chicago" atomizer, 2½ inch mixing chamber. Glycol vaporized from De Vilbiss 180 nebulizer in water bath at 100°C.

Schedule: Same as series II, but with glycol run interposed between control runs.

Series IV.—Psittacosis virus. Runs B14.1 (base control), B14.2 (glycol), B14.3 (second control). June 11, 1945.

Suspension: Strain 6 BC (5Y); yolk sacs 1:10 in extract broth, further dilution 1:500 in extract broth.

Equipment: "Chicago" atomizer, 1 inch mixing chamber. Glycol vaporized from De Vilbiss 180 nebulizer in water bath at 100°C.

Schedule: Cloud samples taken and animals exposed simultaneously for 10 minutes starting 10 minutes after turning on the atomizer. Glycol started 4 minutes before atomizer in run B14.2. Chamber air-washed for 10 minutes between runs 14.2 and 14.3. Atomizer washed by spraying water between runs.

Series V.—Psittacosis virus. Runs B20.1 (base control), B20.2 (glycol), B20.3 (second control), B20.4 (glycol), B20.5 (third control). July 18, 1945.

Suspension: Strain 6 BC (7Y); yolk sacs 1:10 in extract broth, further dilution 1:100 in extract broth.

Equipment: Same as in series IV.

Schedule: Same as in series IV, except that glycol was started 5 minutes before atomizer in runs B20.2 and B20.4, and chamber was air-washed for 15 minutes between runs.

RESULTS

The findings in the three series of experiments with meningopneumonitis virus are summarized in Table II. In the first series (A4), as indicated in Table I, the atomizer reservoir was weighed only before the control and after the glycol run, so that only one value was obtained for fluid output. Hence, although the findings indicate a marked reduction both in cloud recovery and in infective response in exposed mice during the glycol run, the degree to which this reduction may have depended on a decreased atomizer output during the glycol run could not be determined. The result, although suggestive of a positive glycol effect, is thus indecisive.

In the second series an attempt was made to hold the atomizer fluid flow constant by further dilution of the virus in broth, and separate aliquots of the suspension were used for each run so that the concentrations of virus sprayed and recovered could be calculated for each individually (Table I). The first run in this series was a blank control in which air flow to compensate for glycol flow was not used. The other two runs correspond with A4.1 and A4.2. The percentage recovery (Table II) was approximately the same for all three runs, and indicates no reduction in cloud recovered in the glycol run as compared with the air flow control. The lower cloud concentration recovered in these two runs as compared with the blank control, and the correspondingly lower mouse dosage, thus depended in part on the increased total air (or air-vapor) flow, and on the decreased atomizer output (fluid flow) in runs A6.2 and A6.3. The infective response in the mice exposed to virus plus glycol vapor (A6.3) was nevertheless much less than would have been expected from these data.

In all later experiments, done with reconstituted and improved equipment, a control run without glycol was performed *after* a glycol run, in order to com-

pensate for progressive reduction of fluid flow, as well as for any possible diminution of potency of the virus suspension. An initial control run was also included to obtain data uncomplicated by the presence of glycol.

Series III, the last with meningopneumonitis virus, comprised three runs under parallel air flow conditions. Probably because of inadequate dilution of yolk in the virus suspension sprayed, a marked progressive drop in atomizer output occurred (Table I). This fact having been determined immediately after the completion of the runs, the suspensions and samplers were not titrated,

TABLE II

Effect of Triethylene Glycol on Meningopneumonitis Virus

Run No.	Impinger data		Dose LD_{50}*	Animal data								
	Nominal recovery	Reduction in glycol runs		Average No. of pulmonary lesions in mice killed on day						Dead	No. of animals infected /total	Reduction in glycol runs
				4	6	7	8	9	11			Lesion count / Infection
	per cent	*per cent*										*per cent* / *per cent*
A4.1	16.9		3850	30+	37		68				12/12	
A4.2G	2.8	84	630	5	3		1.5				10/12	90 / 17
A6.1	10.6		350	1		19		25	15		16/17	
A6.2	9.6		200	2		4			7		25/26	
A6.3G	9.7	0	190			3			1.5		22/32	78‡ / 27‡
B9.1	—§		—					26		2	6/6	
B9.2G	—		—					7			16/16	55‖ / 0
B9.3	—		—					15			18/18	

* Dosage is estimated in terms of mouse intracerebral LD_{50} units based on sampler titrations.

‡ Estimated by comparison with run A6.2.

§ Samplers were not titrated in this series because of drop in fluid flow.

‖ Estimated by comparison with run B9.3.

and in consequence no data were obtained on the cloud concentration or dosage. The exposed animals were nevertheless maintained and studied. Since the atomizer output in the glycol run was nearly twice as great as that in the second control, it may be assumed that the cloud concentration sprayed was considerably more concentrated in the former. The distinct reduction in infective response in the mice exposed to the glycol cloud therefore seems significant.

The results of the experiments with psittacosis virus are given in Table III. Series IV (Run B14) was performed under conditions similar to those used in series III except that, in an effort to maintain constant atomizer output, the virus was diluted as much as its initial titer seemed to permit, and the atomizer

was washed between runs by spraying water. To this degree the series was successful. The experiment, however, was interrupted on the night preceding the 9th day after exposure of the animals by failure of the vacuum pump in the animal box ventilation line. The death of 16 animals in the second control group (B14.3) is attributed to this accident: postmortem changes made it difficult to count pulmonary lesions in this group and the data are therefore approximate. In this series the cloud concentration recovered in the second

TABLE III

Effect of Triethylene Glycol on Psittacosis Virus

Run No.	Impinger data				Dose LD_{50}*	Animal data		
	Nominal recovery	Average nominal recovery		Reduction in glycol runs		No. of mice dead/total‡	Average pulmonary lesion count§	Reduction of lesion count in glycol runs
		Controls	Glycol					
	per cent	*per cent*	*per cent*	*per cent*				*per cent*
B14.1	28.7				2480	7/18	17.1	
B14.2G	2.1				180	1/20	2.6	
B14.3	7.2	18.0	2.1	88	590	17/20‖	6.8	78¶
B20.1	6.6				8850	3/19	>96	
B20.2G	2.4				3080	0/20	19.6	
B20.3	7.4				9059	2/20	>93	
B20.4G	2.3				2830	0/18	14.5	
B20.5	4.1	6.0	2.3	62	4830	0/19	88	>81¶

* Dosage is estimated in terms of mouse intracerebral LD_{50} units based on sampler titrations.

‡ Includes actual deaths plus mice with massive lung consolidation, in which death is generally predictable in longer experimental periods.

§ Average count per mouse of mice with countable lesions, excluding mice with massive consolidation.

‖ 16 deaths in this group are attributed to failure of animal box ventilation system during the night before the experiment was closed out on the 9th day after exposure.

¶ Estimated by comparison of average counts for glycol and control groups.

control run was distinctly lower than that in the base control, and the mouse dosage was correspondingly lower. Since the fluid flow was held constant within narrow limits, this reduction is believed to be due to insufficient aeration of the chamber between the glycol and second control runs, with consequent persistence of glycol. The results are nevertheless striking. Comparison of the findings in the glycol run with those in either control run shows a clear reduction both in cloud concentration recovered and in infective response in exposed animals.

The complex conditions required for an unequivocal experiment with glycol, determined by experience in the preceding series, were most adequately realized

in series V. This series comprised five runs in which three controls alternated with two glycol runs. The concentration of psittacosis virus sprayed (Table I) was adjusted at a level higher than in series IV in order to induce a more marked response, but low enough to prevent clogging of the atomizer. This step, augmented by washing the atomizer between runs by spraying water, resulted in maintenance of an adequately constant fluid output. The chamber was washed with clean air for 15 minutes between runs in an effort to eliminate the glycol cloud. The results (Table III) show a well marked reduction in the glycol runs as compared with the controls, both in concentration of virus recovered and in response in exposed mice. The recovery in the third control is lower than in the other two, perhaps because of incomplete removal of glycol. The findings nevertheless demonstrate clearly that triethylene glycol, under the conditions employed, exerted a well marked destructive effect on clouds of psittacosis virus.

DISCUSSION

Although only the fifth series of experiments is considered satisfactory in all essentials, the results of all five series of experiments support the general conclusion that triethylene glycol vapor has positive disinfectant value for these virus clouds under the conditions used. These conditions include relative humidity in the range 35 to 45 per cent (or probably higher in the case of meningopneumonitis virus); virus in a dynamic cloud sprayed from suspension in broth; and triethylene glycol concentrations well in excess of saturation. The data do not indicate that these conditions are optimum for a glycol effect. It may be pointed out that they differ from the conditions employed by Robertson and his group, which included the study of static clouds. They differ also from those used by DeOme *et al.* (11), who channeled a dynamic cloud in such a way that the time that elapsed in its passage from atomizer to samplers could be estimated. This procedure is not feasible with the cloud chamber used in the present work. By calculation from the air flow in series V and from the effective volume of the cloud chamber (317 liters), assuming perfect homogeneity of the cloud, it has been estimated that the half-life of the cloud in the chamber was 2.4 minutes (3). This value would represent the mean period of contact of glycol with air-borne virus particles. The concentration of triethylene glycol used in this experiment, having been well in excess of saturation, was considerably greater than those used by previous workers to produce outright sterilization of other agents in shorter intervals. It remains to be determined whether these data point to a discrepancy, or to comparative resistance of psittacosis virus to triethylene glycol, or to differences in experimental conditions.

The findings indicate positive disinfectant action of triethylene glycol on air-borne virus particles, but they also show that enough virus escaped the

action of the disinfectant to induce distinct infection in exposed mice. Insofar as these data bear on the use of triethylene glycol for disinfection of the air of laboratories in which psittacosis virus is handled, they thus indicate that it has definite but limited value. In the absence of information on other means to accomplish the same purpose the use of the vapor is to be recommended, but it is obvious that such use cannot be regarded as providing complete protection, or as an adequate substitute for more direct means to prevent the development of infective aerosols in the laboratory (2).

SUMMARY

1. Triethylene glycol in excess of saturation, introduced as a vapor into a cloud chamber in which a dynamic cloud of either meningopneumonitis virus or psittacosis virus was developed simultaneously with, or in most instances subsequently to, the glycol cloud, induced distinct reduction in concentration of air-borne virus, as measured both by cloud sampling in capillary impingers and by exposure of mice to the cloud. The relative humidity in the chamber was held between 35 and about 60 per cent.

2. Reduction of virus concentration in samplers, in glycol runs as compared with controls, ranged from 0 to 93 per cent (average, 62 per cent).

3. Reduction of infective response in mice, based on counts of focal lesions on lung surfaces, ranged from 55 to 98 per cent (average 73 per cent).

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