No antilysin titers of zero were obtained. This is probably due to interpretation of the results produced by this method. We are unable to obtain a serum control free from antilysin and, therefore, use simply a borate buffer control. We know that lysin is a proteolytic enzyme not specific for fibrin or fibrinogen as it also attacks casein, gelatin, etc. Other proteins in serum may exert minor nonspecific inhibitory effects by competing with the test substrate, fibrin.

Staphylokinase exerted a marked vasodepressor effect which may be associated with some other toxic material in the culture filtrate. Serum lysin showed lesser vasodepressor effects. Guest *et al.*[4] found marked vasodepressor effects following injection of their fibrinolysin[5] which was prepared, by a different method, from bovine serum. These authors found no changes in blood coagulation and did not report any lytic effects. It should be pointed out, however, that our experiment employed a much larger quantity of enzyme.

Some comment should be made on the marked shortening of coagulation time, from 7 minutes to one minute, immediately following administration of the first 50 cc of dog serum lysin. Numerous experiments, in which repeated arterial sampling from a siliconed cannula has been employed to study blood coagulation times in similarly anesthetized dogs, have frequently shown wide variations between 10 and 3½ minutes, with a tendency to become shorter in spite of repeated saline irrigations of the cannula and discarding of the first few cc of blood at each sampling. For this reason we did not feel that the slight shortenings of clotting-time observed after injection of staphylokinase were significant. On the other hand, the marked drop in clotting-time following injection of serum lysin cannot be dismissed as an experimental error. This is possibly due to the effects of small amounts of thrombin, which were present as a contaminant of this preparation of dog serum lysin.

Conclusions. 1. Staphylokinase, injected intravenously into a dog, caused appearance of active serum lysin, accompanied by a fall in prolysin and antilysin. The blood became incoagulable presumably due to lysis of the circulating fibrinogen. A precipitous fall in blood pressure was also observed.

2. Dog serum lysin, injected intravenously into a dog, also resulted in the appearance of active serum lysin, fall in antilysin, but no appreciable change in prolysin. Incoagulable blood, devoid of fibrinogen, was again obtained.

[4] Guest, M. M., Murphy, R. C., Bodnar, S. R., Ware, A. G., and Seegers, W. H., *Am. J. Physiol.*, 1947, **150**, 471.

[5] Loomis, E. C., George, J. C., and Ryder A., *Arch. Biochem.*, 1947, **12**, 1.

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17298. Lethal Effect of Triethylene Glycol Vapor on Air-Borne Mumps Virus and Newcastle Disease Virus.*

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Previous laboratory investigations by Robertson and his co-workers,[1,2] and by Henle and Zellat[3] demonstrated that both propylene and triethylene glycol vapor were lethal for air-borne Influenza A virus. Subsequently, Rosebury and his associates[4] showed that triethylene glycol vapor was effective against air-borne meningopneumonitis and psittacosis

* Aided by grants from the U. S. Public Health Service, Carbide and Carbon Chemicals Corporation, and the Research Corporation.

† National Institutes of Health Postdoctorate Research Fellow.

[1] Robertson, O. H., Loosli, C. G., Puck, T. T., Bigg, E., and Miller, B. F., *Science*, 1941, **94**, 612.

[2] Robertson, O. H., Puck, T. T., Lemon, H. F., Loosli, C. G., *Science*, 1943, **97**, 142.

virus. These observations indicated that the glycol vapors were virucidal as well as bactericidal.[2,5] The experiments to be described demonstrate that triethylene glycol vapor is lethal for two more viral agents, mumps virus and Newcastle disease virus.

Materials and methods. Viruses. A strain of mumps virus‡ was cultivated in the allantoic sac of 9 day old chick embryos which, after inoculation, were incubated at 35°C for 6 days. The infected embryos were then chilled over night at 4°C and the allantoic fluid removed.

The strain of Newcastle disease virus‡ was cultivated in the allantoic sac of 11 day old embryos, which after inoculation, were incubated at 37°C for 52 hours. Infected allantoic fluid was harvested after chilling overnight at 4°C.

Virus Infectivity Titrations. These were performed on the infected allantoic fluids which were to be sprayed into the chamber. Serial tenfold dilutions from 10^{-5} to 10^{-10} were made in sterile broth. A volume of 0.1 cc of the dilution of virus was inoculated through a small drill hole in the egg shell directly over the allantoic sac. Four embryos were used for each virus dilution. Each allantoic fluid was tested for its capacity to agglutinate chicken erythrocytes using the method of Salk.[6] Virus titration end points (E.I. 50)§ were calculated by the 50% end point method of Reed and Muench.[7]

The Glycol Chamber.‖, constructed of wood and glass, (Fig. 1), had a capacity of 5.5 cubic feet. The air was gently circulated by

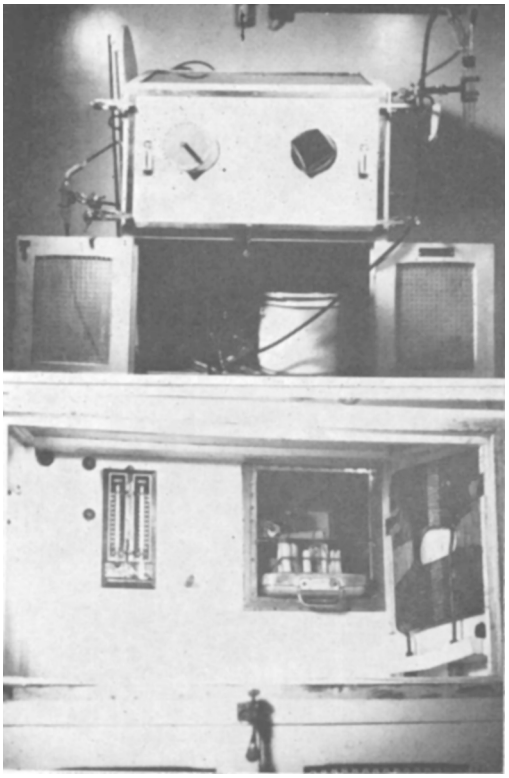

FIG. 1.

a small fan in the left rear compartment. In front of the fan was a Screw Base Resistance Element (10 watts) which served as a source of heat to vaporize glycol impregnated paper. On the left wall of the chamber there were a series of circular coils through which ice water was circulated by means of an electrical water pump. This cooling apparatus was controlled by a thermostat set at the desired temperature.

Viral suspensions were atomized into the chamber through a port on the right side. Air bubbler samples were taken through a port on the left side. A measured volume of air, as indicated by a flowmeter,[8] was withdrawn into a suitable medium for trapping the infective agent. A Welch air pump was used to

[3] Henle, W., and Zellat, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 544.

[4] Rosebury, T., Meiklejohn, G., Kingsland, L. C., and Boldt, M. H., *J. Exp. Med.*, 1947, **85**, 65.

[5] Robertson, O. H., Bigg, E., Puck, T. T., and Miller, B. F., *J. Exp. Med.*, 1942, **75**, 593.

‡ We are indebted to Dr. Frank L. Horsfall, Jr., of the Rockefeller Institute for Medical Research, for the allantoic strain of mumps virus, and to Dr. Alfred S. Evans of Yale University, for the Newcastle disease virus.

[6] Salk, J. E., *J. Immunol.*, 1944, **49**, 87.

§ 50% embryo infectivity end point.

[7] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

‖ We are indebted to Dr. Charles C. Chapple of Philadelphia and to Mr. J. W. Spiezelman of Air Purification Service, Inc., for designing the Glycol Chamber.

[8] Lemon, H. M., and Wise, H., *Science*, 1944, **99**, 43.

provide positive pressure for atomization of the suspension and negative pressure for withdrawal of air through a bubbler sampler.[9]

The front partition of the chamber was removable and had two circular perforations 5 inches in diameter. The one on the right was sealed with a cylindrical double air lock which could permit the entry of animals of the size of guinea pigs. The one on the left was sealed by a large rubber gloved sleeve which extended into the chamber, and which could be used for exposing settling plates at periodic intervals.

Triethylene glycol was vaporized into this chamber by placing a strip of impregnated paper on the heating element. This paper¶ contained 0.03 g of triethylene glycol per square inch. The glycol vaporized rapidly and saturated the air of the chamber.

A hygrometer consisting of a dry bulb and wet bulb was located on the back wall of the chamber. The air stream produced by the fan passed over the wet bulb and thus a fairly accurate determination of relative humidity was obtained.

Experimental. Preliminary experiments were conducted to determine if mumps virus could be rendered air-borne, and if so, for how long it could be recovered from the air. Infected allantoic fluid was sprayed into the chamber. Immediately after cessation of spray, serial air bubbler samples were taken for a 15 minute period. An aliquot of each sample was inoculated into the allantoic cavity of 9 day old chick embryos. After 6 days of incubation at 35°C. the fluid was harvested following a preliminary period chilling at 4°C. Each fluid was then tested for its capacity to agglutinate chicken erythrocytes. The results of this procedure indicated that mumps virus could be recovered from the air of the chamber for at least 12 minutes after cessation of spray. This was considered to be an adequate control, and the following experiments with triethylene glycol vapor were then performed.

Effect of Triethylene Glycol Vapor on Air-Borne Mumps Virus. Control Test. 2 cc of allantoic fluid with an E.I. 50 titer of 10^{-8} was delivered by a Graeser atomizer**[10] attached to the inlet port of the chamber. Immediately after cessation of the spray, 2 minute serial air bubbler samples were withdrawn for a 15 minute period. Each bubbler contained 10 cc of 10% horse serum in neopeptone broth. After sampling, each bubbler was treated with penicillin (500 U/cc) and streptomycin (3 mg/cc). An aliquot (0.1 cc) of each sample was inoculated intra-allantoically into 4 chick embryos. After 6 days of incubation at 35°C, the allantoic fluids were harvested. Hemagglutination titrations were then performed on the individual allantoic fluids.

Glycol Test. After completion of the control test the chamber was saturated with triethylene glycol vapor. The identical procedure was then repeated.

Environmental Conditions. The temperature was 83°F (28°C) and the relative humidity was 36% in both the control and glycol tests.

Results. Mumps virus was obtained from the air of the control chamber for at least 13 minutes after cessation of spray. When the air of the chamber was saturated with triethylene glycol vapor, no mumps virus could be recovered (Table I). In 2 of 4 experiments no virus could be detected even immediately after cessation of spray. In the other 2 confirmatory experiments, virus was recovered only in the first sample taken immediately after completion of spray, but at no time thereafter.

In order to determine whether negative hemagglutination represented absence of virus or low titer virus, the following procedure was carried out. The negative allantoic fluids of each sample were pooled and each pool was inoculated intra-allantoically into 4 chick embryos. After incubation and harvesting, the individual allantoic fluids were tested for their capacity to agglutinate chicken erythro-

[9] Lemon, H. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 298.

¶ Prepared by Air Purification Service, Inc., Newark, N. J.

** Delivers a particle size of 1.7 μ mean mass diameter.

[10] Graeser, J. B., and Rowe, A. H., *Am. J. Dis. Child.*, 1936, **52**, 92.

TABLE I.
Effect of Triethylene Glycol Vapor on Air-Borne Mumps Virus.

Time interval min. after spray	Control test Hemagglutination titer* Individual allantoic fluids				Mean	Glycol test Hemagglutination titer* Individual allantoic fluids				Mean
0-2	5120	5120	5120	160	2560	0	0	0	0	0
3-5	160	160	160	640	240	0	0	0	0	0
6-8	160	0	640	80	80	0	0	0	0	0
10-12	0	320	0	1280	60	0	0	0	0	0
13-15	0	1280	0	80	40	0	0	0	0	0

* Expressed as reciprocal.

TABLE II.
Effect of Triethylene Glycol Vapor on Air-Borne Newcastle Disease Virus.

Time interval min. after spray	Control test Hemagglutination titer* Individual allantoic fluids				Mean	Glycol test Hemagglutination titer* Individual allantoic fluids				Mean
0-½	5120	5120	1280	5120	3840	0	0	0	0	0
1-1½	5120	5120	5120	—	5120	0	0	0	0	0
2-2½	5120	5120	1280	5120	3840	0	0	0	0	0
5-5½	5120	5120	5120	5120	5120	0	0	0	0	0
10-10½	640	0	5120	—	265	—	—	—	—	—
15-15½	0	0	0	—	0	—	—	—	—	—

* Expressed as reciprocal.
— Not done.

cytes. This hemagglutination test was completely negative and was interpreted as indicating absence of virus rather than low titer virus.

In the first experiment with mumps virus, the agent was detected in all samples during the 15 minutes control period. In the presence of a saturated atmosphere of triethylene glycol vapor, no virus could be recovered. The chamber was then cleaned and aired for a 2 week period. When the same experiment was repeated, mumps virus could only be recovered from the first (0-2 min.) sample in the control test. It seemed possible that the minute amount of residual glycol, which had condensed on the wall of the chamber, was sufficient to interfere with the control test. There was no visible vapor and one can only guess that the percentage saturation of triethylene glycol vapor in the chamber air was very low. After heating the interior of the chamber with a 200 watt bulb to vaporize all of the residual glycol, the original experiment was easily repeated and confirmed. These observations suggest that triethylene glycol vapor is lethal for mumps virus in concentrations below saturation.

Effect of Triethylene Glycol Vapor on Air-Borne Newcastle Disease Virus. The virus infectivity titration of the allantoic fluid was 10^{-8} E.I. 50. This material was used for a control test and a glycol test. The procedure was the same as that used in the mumps experiments with the following modifications: 1) 1½ cc of Newcastle disease virus was atomized into the chamber. 2) ½ minute air bubbler samples were taken. 3) Each bubbler contained 8 cc of 1% Casamino acids. 4) The temperature was 74°F (23°C) and the relative humidity was 50% during both the control and glycol tests.

Results. As indicated in Table II, Newcastle disease virus could be obtained from the air of the control chamber for 10 minutes after cessation of spray. No virus could be recovered when the chamber was saturated with triethylene glycol vapor.

Discussion. In this study, the hemagglutination test has been used as a measure of virus concentration. Beveridge and Lind[12] found that there was a direct relation between

[12] Beveridge, W. I. B., and Lind, P. E., *Australian J. Exp. Biol. and Med. Sc.*, 1946, **24**, 127.

the hemagglutination and complement fixation titers of mumps infected allantoic and amniotic fluids and concluded that the hemagglutination titer was a function of virus concentration. Ginsberg, Goebel and Horsfall[11] showed that the rate of increase in concentration of virus, as determined by infectivity titrations, paralleled the rise of hemagglutination titers. The latter workers also found that an E.I. 50 titer of the order of $10^{-4.30}$ or higher was necessary before hemagglutination with mumps virus was demonstrable.

In order to rule out the possibility of a virus concentration titer too low to be detected by hemagglutination, the allantoic fluids of the glycolized air samples were reinoculated into chick embryos. Following this second passage, no rise in titer was observed, and it was therefore concluded that no mumps virus was present.

Summary. 1. Under the conditions of these experiments, triethylene glycol vapor in saturated concentrations was rapidly lethal for air-borne mumps virus and Newcastle disease virus.

2. Triethylene glycol vapor was also lethal for air-borne mumps virus in concentrations below saturation.

We should like to express our indebtedness to Dr. Robert Ward for his helpful advice throughout this study.

11 Ginsberg, H. S., Goebel, W. F., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1948, **87**, 385.

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17299. Effect of Ammonium Chloride on Serum Sodium/Chloride Ratio in Foreign Serum Arteritis in Rabbits.

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An arteritis has been induced in rats by the administration of large amounts of desoxycorticosterone acetate (D.C.A.) by Selye,[1] and in rats so treated the development of these lesions was inhibited when NH_4Cl was added to their diet.[1] D.C.A. overdosage in rats also causes a rise in the serum sodium and the serum Na/Cl ratio[1,2] and a reduction of this ratio toward the norm following NH_4Cl therapy has been held to be significant in inhibiting the development of D.C.A. induced arteritis and other lesions in the rat.[1] It was thought that if this elevation of the serum Na/Cl ratio was an essential step in the pathogenesis of the D.C.A. arteritis, then if there is a common pathogenesis in the development of the D.C.A. arteritis and the well known foreign protein arteritis,[3] there should also be an elevation of the serum sodium and Na/Cl ratio in animals given foreign protein which develop arteritis. It also seemed a logical possibility that ammonium chloride might inhibit the development of foreign protein arteritis if there is a common pathogenesis in the development of these two types of arteritis. The following experiments were designed to determine whether these logical possibilities concerning the pathogenesis of these two types of arteritis are correct.

Materials and methods. Group 1. Eighteen albino rabbits of 2-3 kg weight were given a diet of Purina Rabbit Chow and water to drink *ad libitum*. The rabbits were given an intravenous injection of sterile whole horse serum in a dose of 10 cc/kg of body weight. The serum injection was repeated after 17 days. Seven days later all animals were killed by air embolism and sections were prepared for histological study from all tissues as in our previous studies.[3]

Group 2. Seventeen albino rabbits of 2-3 kg weight derived from the same sources and

1 Selye, H., Hall, O., and Rowley, E. M., *Lancet*, 1945, **248**, 301.

2 Friedman, S. M., Polley, J. R., and Friedman, C. L., *J. Exp. Med.*, 1948, **87**, 329.

3 More, R. H., and McLean, C. R., *Am. J. Path.*, 1949, **25**, 413.