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Viricidal activity of open air

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SUMMARY

Airborne Semliki Forest virus and T coliphages were inactivated at a considerably enhanced rate in open air compared with enclosed air. Open air exerts its maximum sterilizing activity on viruses contained in the smallest sized particles.

INTRODUCTION

It is well known that viruses can remain infectious for long periods in airborne particles (Harper, 1961, 1963; De Jong & Winkler, 1964, 1968; Akers, Bond & Goldberg, 1966; Songer, 1967; Benbough, 1969, 1971). These reports show that the two principal factors that affect virus inactivation rates in the aerosol are the relative humidity of the air surrounding the infectious particles and the nature and concentration of solute content in the fluid from which the particles are generated. These results were obtained from experiments conducted in enclosed atmospheres and may therefore be of doubtful application in epidemiology. What is needed is a technique for estimating virus survival in ordinary outside air. This is difficult because stringent precautions against contaminating the habited environment must be maintained. The bactericidal activity of open air has been demonstrated by the microthread technique (May & Druett, 1968) and by the ventilated sphere technique (Hood, 1971). These methods have been used here to determine the viricidal activity of open air and compare it with bactericidal activity. The bactericidal activity has been attributed to forms of atmospheric pollution (Druett & May, 1968) and recently it has been postulated that the bactericidal agent in outside air is a gaseous complex formed by the combination of ozone, normally present in air, and gaseous unsaturated hydrocarbons such as those freely generated from internal combustion engines (Druett & Packman, 1968; Dark & Nash, 1970). It has been shown that the bactericidal activity of open air is rapidly lost during enclosure of the air; this is presumably due to the adsorption of the gaseous complex to the walls of the container (May & Druett, 1968).

The ventilated-sphere technique (Hood, 1971) has enabled us to determine the inactivation rates of viruses in open air as a function of the diameter of the particles in which the viruses are located. We consider this to be an important contribution since studies on the virus survival in particles of different size would be useful in assessing the susceptibility of animals to infection. Open wounds and

the upper parts of the respiratory system are expected to be more vulnerable to infection by organisms contained in large particles, whereas the pulmonary alveoli are accessible only to organisms contained in the smallest particles.

METHODS

Semliki Forest virus and coliphages T1 and T7 were grown as described before (Benbough, 1971). *Escherichia coli* MRE 162 was grown as described by Cox (1966). *Bacillus subtilis* var. *niger* spores were used as a tracer to allow for the physical decay of aerosols. The suspending fluids and the microbial assays have been described before (Cox, 1966; Benbough, 1971).

The microthread technique

The aerosols generated by a Collison spray and deposited on microthreads (May & Druett, 1968) were exposed to the open air and shaded from direct sunlight. Microthreads to which arbovirus was attached were kept near the mouth of a duct through which open air was drawn (May, Druett & Packman, 1969). This is a device used when potentially pathogenic micro-organisms were tested.

The ventilated sphere

This system has been used to determine the survival of microbes in true aerosols in open air. The sphere itself is a metal chamber of 22 ft. diameter from which aerosol is sampled by means of a tubular appendage, the whole system being usually operated in a closed state. In its ventilated role outside air is drawn through the sphere at such a rate that loss of germicidal activity due to enclosure is overcome. The minimum ventilation rate required to achieve this is 12 air changes/hr. and in practice the rate used is 14 changes/hr. The rates apply to tests of both bacteria and viruses.

A suspension containing approximately 2×10^{11} p.f.u. of the test virus, 2×10^{11} *Bacillus subtilis* spores and 2·4% (w/v) solutes in 200 ml. of fluid was completely aerosolized within 5 min. by means of a fluid atomizer (May, 1966) at the centre of the sphere. A wide range of particle sizes are produced by this spray. Aerosol samples were taken at intervals using the three-stage liquid impingers (May, 1966) which separate aerosol particles into three fractions according to size ($< 3\,\mu$m., 3–6 μm. and $> 6\,\mu$m. in diameter).

Usually the viricidal effect of open air was tested by microthreads and the sphere concurrently. For the purpose of comparison with bactericidal activity the survival of *Escherichia coli* MRE 162 on microthreads exposed to the same air was measured.

RESULTS

Microthread tests

The decay rates of *E. coli* MRE 162 held on microthreads and exposed to open air varied enormously from day to day (Fig. 1). These decay rates ranged from 0·3% min.$^{-1}$ to 5·0% min.$^{-1}$ over a 1 hr. exposure period in relative humidities

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between 75% and 96% and temperatures between 2° and 12° C. The decay rate in enclosed atmospheres in these conditions never exceeded 0·2% min.$^{-1}$.

T1 and T7 coliphages on microthreads also had enhanced decay rates in open air compared to enclosed air. However, unlike those of bacteria, decay rates of the coliphages did not vary greatly from day to day. For example, when T1 coliphage was exposed on 40 different days to open air the decay rates were within the range of 1·0% min.$^{-1}$ to 1·4% min.$^{-1}$ for a 1 hr. exposure period (Fig. 2*a*). A similar type of result was found when T7 coliphage was tested (Fig. 2*b*).

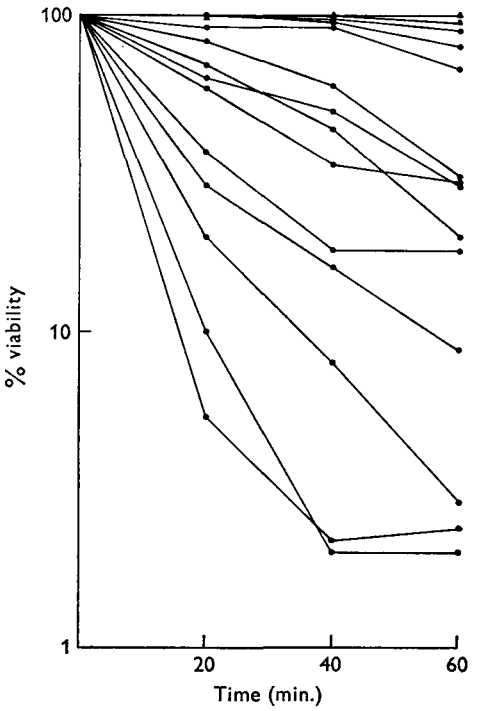

Fig. 1. The decay rates of *Escherichia coli* MRE 162 attached to microthreads and exposed to the open air on different days (all experiments were conducted at temperatures from 2° to 12° C. and relative humidity 75–96%). ▲, Microthreads in enclosed air; ●, microthreads exposed to open air.

When Semliki Forest virus was held on microthreads inactivation of the virus occurred at a rate of 2% min.$^{-1}$ even in enclosed air at relative humidities of 75% and above. This is atypical of the survival characteristics of Semliki Forest virus in true airborne particles. For example, in a rotating drum at these conditions, the rate of inactivation did not exceed 0·1% min.$^{-1}$ (Benbough, 1969, 1971, also see later for non-ventilated sphere data). Therefore, the microthread technique cannot be considered ideal for simulating Semliki Forest virus in true aerosols. Nevertheless experiments which were done using microthreads showed that Semliki Forest virus was inactivated at a much faster rate in open air (10% min.$^{-1}$) than in enclosed air (2% min.$^{-1}$) (Fig. 3). Again it was found that the day-to-day variation in the inactivation rates in open air was small.

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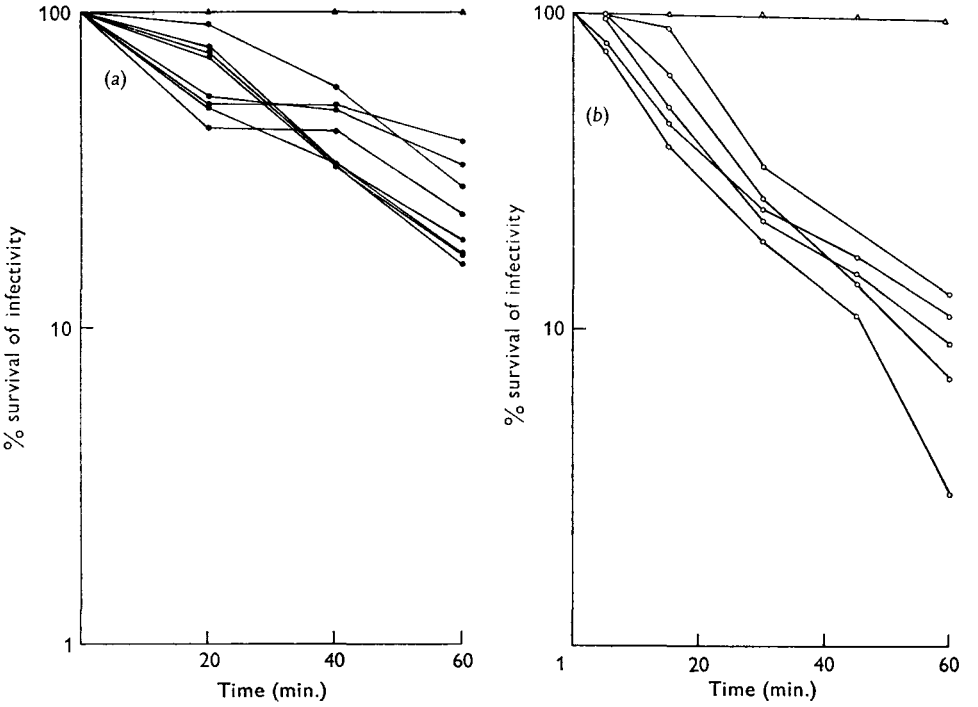

Fig. 2(*a*). The inactivation rates of T1 coliphage attached to microthreads and exposed to open air (all experiments were conducted at temperatures from 2° to 12° C. and relative humidity 75–96%). ▲, Microthreads in enclosed air; ●, microthreads exposed to open air. (*b*) The inactivation rates of T7 coliphage attached to microthreads and exposed to open air (between 2° and 12° C. and 75–96% relative humidity). △, Microthreads in enclosed air; ○, microthreads exposed to open air.

Ventilated-sphere tests

Because the microthread technique is inexplicably unsuitable for simulating aerosols containing Semliki Forest virus, the use of the sphere to measure the viricidal activity of open air assumes greater importance. Many tests (20) conducted in the ventilated sphere showed that the rate of Semliki Forest virus inactivation ranged from 1·3% min.$^{-1}$ to 1·5% min.$^{-1}$ in aerosols at relative humidities between 75% and 95% and temperatures between 2° and 12° C. Over the same storage period in the closed (non-ventilated) sphere the inactivation of Semliki Forest virus is approximately 0·1% min.$^{-1}$. Therefore the ventilated sphere experiments show a considerable viricidal activity of open air which is fairly constant from day to day (Fig. 4). The results also show that the inactivation rate of Semliki Forest virus has an inverse relationship to the diameter of the airborne particle in which the virus is contained. Fig. 5 shows that this relationship also applies to airborne T1 coliphage.

In Fig. 6 is shown the decay rates of *E. coli* MRE 162 (measured on microthreads) and of Semliki Forest virus (in the ventilated sphere) plotted against each other

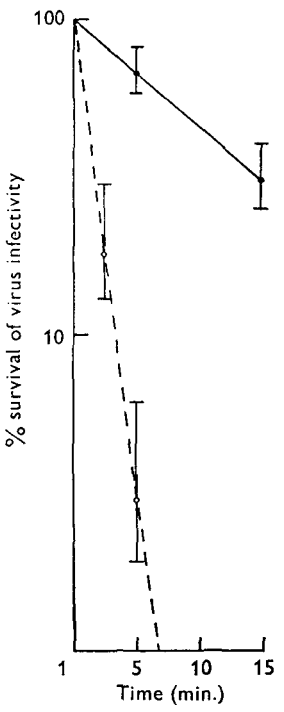

Fig. 3

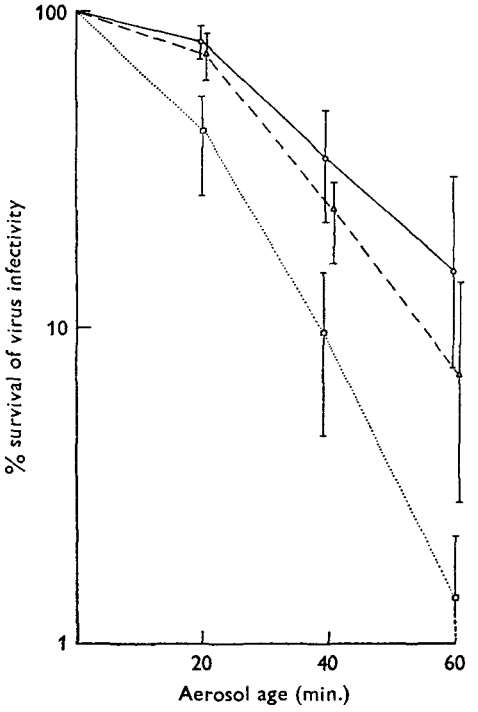

Fig. 4

Fig. 3. The inactivation of Semliki Forest virus on microthreads. ●, In enclosed air; ○, in open air.

Fig. 4. The inactivation of Semliki Forest virus in different sized particles in the ventilated sphere. ○, Virus contained in particles over 6 μ in diameter; △, particles between 6μ and 3μ in diameter; □, particles less than 3 μ in diameter. Each point is an average of 20 experiments carried out on different days at temperatures from 2° to 12° C. and relative humidities from 75 to 96 %.

for each day that both were exposed. This plot shows that the viricidal activity is constant from day to day whereas the bactericidal activity varies considerably.

DISCUSSION

The large day-to-day differences in the decay rates of airborne *E. coli* MRE 162 could be accounted for by large variations in the concentration of an unknown bactericidal atmospheric pollutant (May & Druett, 1968; Dark & Nash, 1970). The concentration of pollutant surrounding the bacteria under test will depend upon the distance between the pollutant source and test areas and the direction of the wind. Also, if the pollutant is unstable the wind speed between the source and test areas would be important. The constant viricidal activity of open air suggests the possibility that this is caused by another pollutant whose concentration is constant regardless of the meteorological conditions.

Alternatively airborne viruses and bacteria may be inactivated by the same component in open air but may be susceptible to different concentration ranges.

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Fig. 7 shows the kind of response to be expected if bacterial decay gradually increases with increase in concentration of airborne pollutant whereas maximum virus decay is caused by traces of the same component that are invariably present in open air.

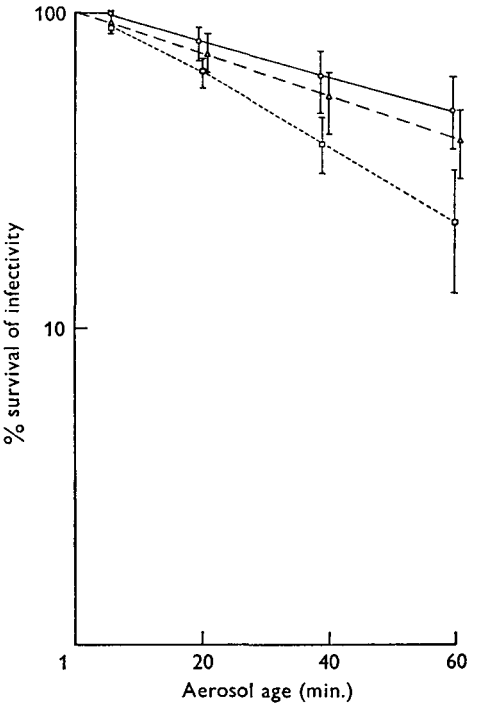

Fig. 5. The inactivation of T 1 coliphage in different-sized particles in the ventilated sphere. ○, Phage in particles over 6μ diameter; △, in particles between 6 μ and 3 μ; □, in particles less than 3 μ. Each point represents an average of four experiments on different days at temperatures between 7° and 12° C. and relative humidities between 78 % and 92 %.

The ventilated-sphere method has yielded data on the survival of viruses in particles of different sizes that have been previously lacking in the literature. In enclosed air no differences in infectivity related to particle size can be detected. In open-air conditions a particle size effect is observed. If the airborne component attacks through the surface of particles containing virus then the simplest hypothesis is that virus inactivation rate is directly proportional to the surface to volume ratio of the particle; that is, $\pi d^2/\pi d^3/6$, which is to the inverse of the particle diameter, d.

The marked viricidal activity of open air alters predictions for survivals of normally occurring animal viruses in open air. For example, the airborne route has been strongly implicated as the cause of cross-infection of foot-and-mouth disease virus (Hyslop, 1965; Henderson, 1969; Sellers & Parker, 1969). In enclosed air this virus behaves in the aerosol like poliovirus (De Jong & Winkler, 1968), Columbia SK group virus (Akers *et al.* 1966) and T-coliphages (Benbough, 1971)

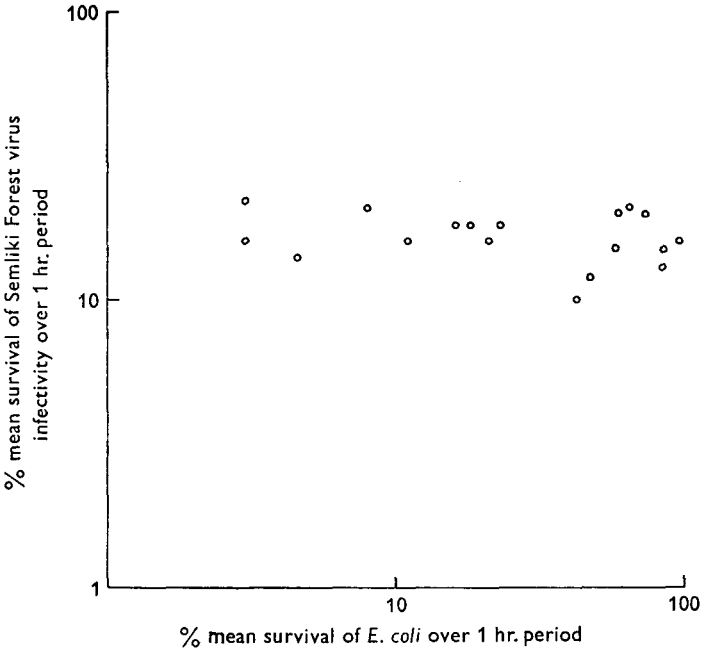

Fig. 6. The variable bactericidal effect compared to the constant viricidal effect of open air on different days. (The % mean survival over 1 hr. is defined as the mean survival of organisms at 20 min., 40 min. and 60 min.)

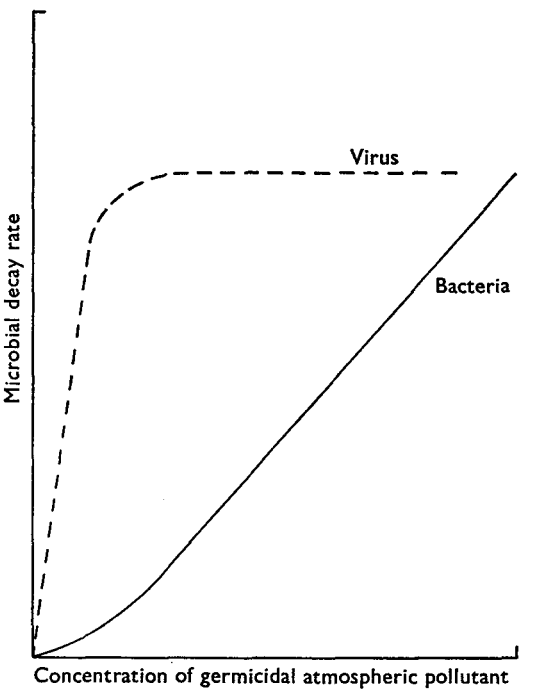

Fig. 7. The two different possible responses of the decay rates of airborne bacteria and viruses to concentration of atmospheric pollutants.

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in that it survives well only at relative humidities of 70% or over (G. J. Harper, personal communication). The viricidal activity of the agent present in open air may alter Sellers & Parker's (1969) calculation on the persistence of infective foot-and-mouth virus downwind in cold humid conditions by a considerable amount. Assuming the susceptibility of this virus's infectivity to the agent to be similar to viruses tested here, then its persistence in similar conditions would be considerably lowered.

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