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# Open-air factors in enclosed systems

By A. M. HOOD
*Microbiological Research Establishment, Porton Down, Salisbury, Wilts.*

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## SUMMARY

Ventilation of vessels varying widely in size was found to preserve the toxic effect of open-air factor(s). There was a correlation between the minimum rates of ventilation required and the ratios of the surface area of the vessels to their volumes. The data obtained allowed an estimate to be made of the diffusion coefficient of open-air factor(s) and gave an indication that the molecular weight range of the open air factor(s) was from 50 to 150.

## INTRODUCTION

Open-air factors (OAF) which decrease microbial survival usually disappear when the air is enclosed (Druett & May, 1968; Hood, 1971). Their effect therefore cannot be studied in conventional laboratory apparatus. To overcome this limitation the ventilated sphere system was developed (Hood, 1971). Success with this system suggested that it might be worth while examining the ephemeral character of OAF further in different vessels covering a wide range of sizes. The experimental vessels could be ventilated at rates that enabled the toxic properties of open air to be fully preserved. The minimum ventilation rate was found to be proportional to the ratio of the surface area of the vessel to its volume. The data obtained allowed an estimate to be made of the diffusion coefficient of OAF and hence gave an indication of its molecular weight.

## MATERIALS AND METHODS

Air toxicity was measured biologically by observing the survival of *E. coli* MRE 162 (EC) exposed in small particles ($< 3\ \mu$m. diam.) attached to microthreads (May & Druett, 1968) as described previously (Hood, 1971). Conditions of temperature and relative humidity were chosen for which, in 'clean' air in enclosed systems, the *E. coli* was relatively unaffected for at least 2 hr., i.e. within the relative humidity range of 70–100% at ambient temperatures. Thus the decrease in viability of the organisms exposed concurrently to open air and to the air in ventilated systems could be attributed solely to OAF. The mean survival of *E. coli* (the arithmetic mean of the viabilities measured at several 15 or 20 min. intervals during the total exposure period) was obtained in open air and in the ventilated systems. The ratio of the survival of the bacteria in open air to its survival in air in ventilated systems was used as a relative potency index to compare the toxicity

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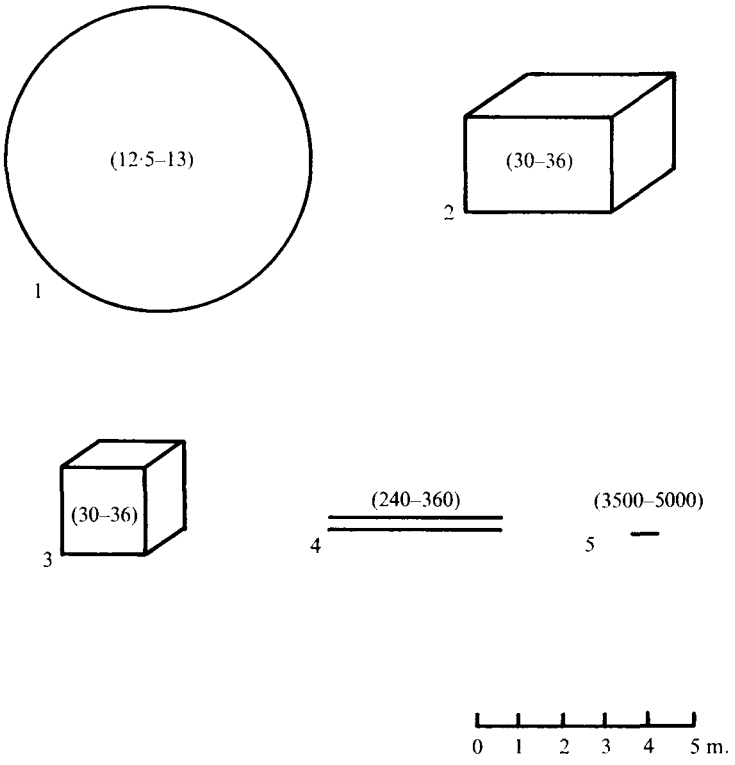


Fig. 1. Ventilated vessels used. 1, Sphere, 7 m. diameter; 2, cuboid, 3·43 × 2·28 × 2·28 m.; 3, cube, 2 × 2 × 2 m.; 4, tube, 4·6 m. × 0·35 m. diameter; 5, tube ('sow') 0·4 × 0·06 × 0·04 m. Figures in parentheses indicate minimum ventilation rate ranges (air changes/hr.) to obtain fully bactericidal effect of open-air factors.

of air in vessels ventilated at various rates and at various times with the toxicity of open air (which varied from day to day).

A maximum of $1\frac{1}{2}$ hr. exposure was used. In order to reflect the most significant data, only results obtained from those occasions when open air caused over 80% loss of viability in this time are presented.

The known effects of daylight and air velocity on viability were minimized by exposing the open-air microthreads in a 'roundabout' (Druett & May, 1969) on the lee-side of a large building.

The minimum ventilation rate which apparently fully preserved the toxicity of open air was determined for each of five vessels: a sphere (7 m. diam.), a cuboid (3·43 × 2·28 × 2·28 m.), a cube (2·2 m.), a tube (4·6 × 0·35 m. diam.), and a microthread loading 'sow' (May & Druett, 1968) about 0·4 m. in length and 24 cm.$^2$ cross-section. They were made of mild steel, aluminium, mild steel painted, stainless steel and brass respectively and are shown to scale in Fig. 1.

Since the *E. coli* were being exposed concurrently on microthreads in situations in which the air velocity was not always the same, preliminary tests were made to determine the degree to which air velocity would affect the comparisons.

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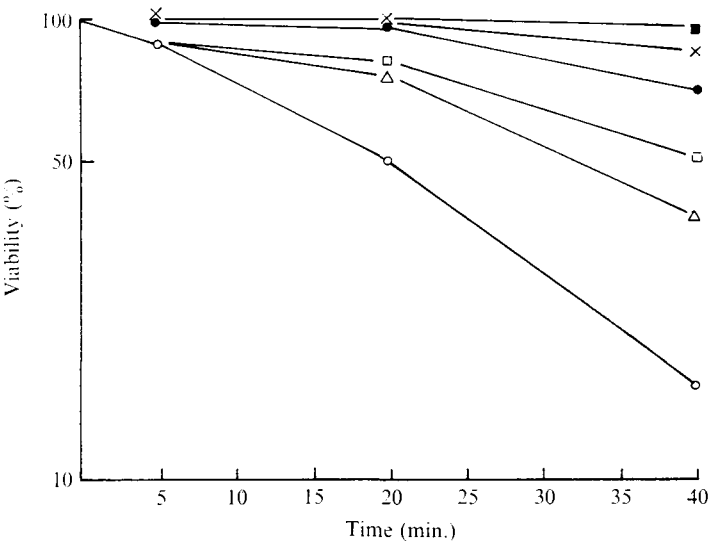


Fig. 2. Air velocity effect on viability of *Escherichia coli* held on microthread. ■, Indoor air at 0 to 1·4 m./sec.; ×, indoor air at 2·8 m./sec.; ●, indoor air at 5·7 m./sec.; □, open air at 1·4 m./sec.; △, open air at 2·8 m./sec.; ○, open air at 5·7 m./sec.

## RESULTS

### *Air velocity effect on viability of* E. coli *exposed on microthreads*

The effect of air velocity was tested using a whirling arm similar to that described by May & Druett (1968). The suspending medium and relative humidity range, however, were different. Tests were made at *ca.* 0, 1·4, 2·8 and 5·7 m.sec.$^{-1}$ air velocities in enclosed ('clean') air and in open air. In clean air no adverse effect on viability was apparent at air velocities below 5·7 m.sec.$^{-1}$. At 5·7 m.sec.$^{-1}$ there was a small – possibly significant – effect on survival. In OAF conditions there was a slight air velocity effect at 2·8 m.sec.$^{-1}$ and this increased considerably at 5·7 m.sec.$^{-1}$ (Fig. 2).

The results indicated that the contribution made by air velocity to the viable decay of *E. coli* would not be significant in ventilated vessels in which the air velocity did not exceed 2·8 m.sec.$^{-1}$ when the air was 'clean'. In air containing OAF, velocities in excess of 1·4 m.sec.$^{-1}$ could make a significant contribution to the death-rate. It was not found necessary to exceed these limits in the ventilated vessels and unlikely under the chosen site conditions for exposure in open air. Erroneous comparisons between air in the ventilated systems and open air due to air velocity effect were thus considered unlikely and in any event would be small.

### *OAF persistence in ventilated vessels*

The results obtained at various rates of ventilation in each of the five vessels are shown in Fig. 3. The minimum ventilation rates (air changes/hr.) which apparently preserved open-air toxicity in full were as follows: sphere, 12·5–13; cuboid and cube, 30–36; tube, 240–360; and 'sow', 3500–5000. Consistent results

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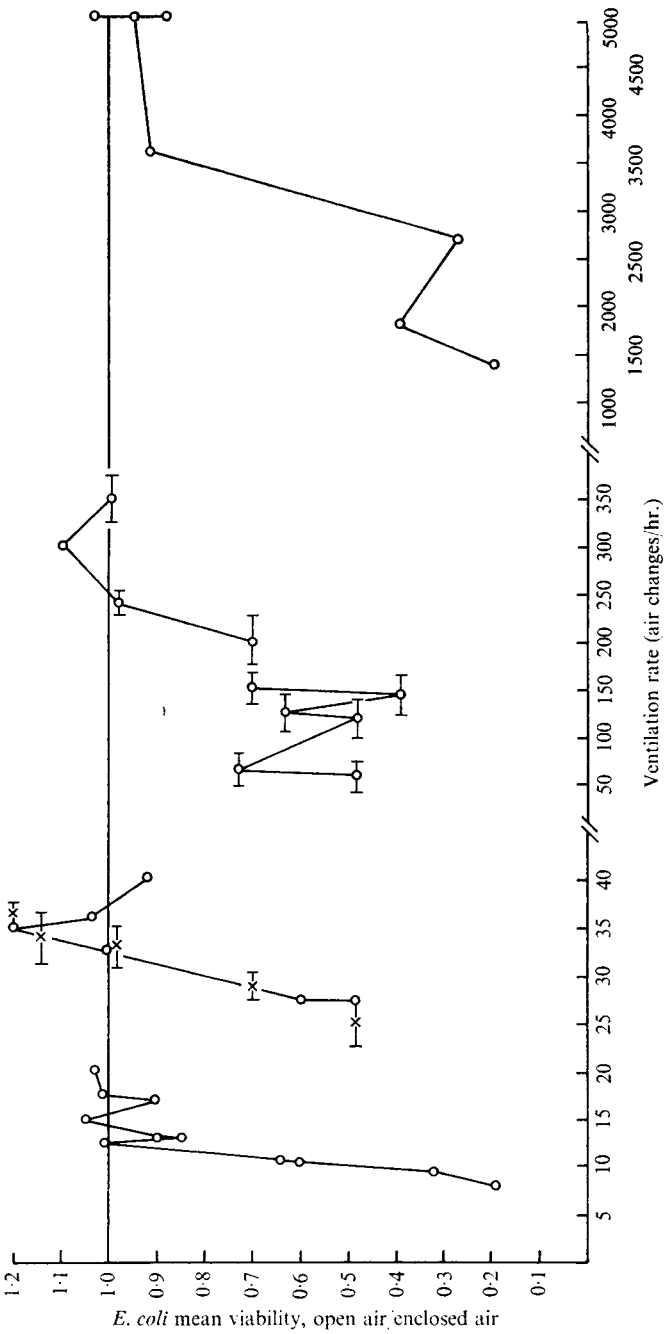


Fig. 3. Open-air factors in vessels ventilated at various rates. 1, Sphere; 2, (○) cuboid; 3, (×) cube; 4, tube; 5, 'sow'.

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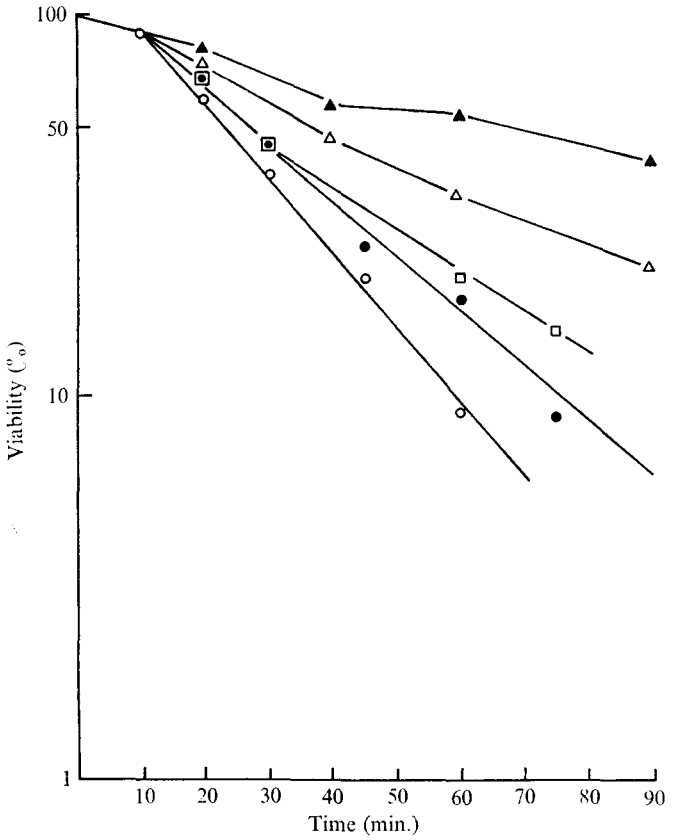


Fig. 4. *Escherichia coli* response to various concentrations of open air. ○, 100% open air; ●, 95%; □, 90%; △, 75%; ▲, 50%.

could only be obtained in the larger vessels when fans were used to ensure adequate mixing of the air within them. The tube was examined by comparing air at the extract end with that at the open (air intake) end. It was not possible to obtain narrower limits of ventilation rates because of fluctuation of air flow caused by ambient winds with this tube when used at the low flow rates found to be required to maintain the OAF throughout its length. The 'sow' was ventilated by application of a negative pressure through critical orifices of a range to give the desired flow and hence ventilation rates.

*Biological response to OAF*

The results described above show that each vessel can be ventilated at such a rate that there is no apparent loss of the potency of open air. In order to establish a quantitative correlation, however, it is necessary to determine the relationship between the biological response and the OAF concentration. The results obtained with the 'sow' suggested a system that could be used for such a study. Sows were ventilated with open air, or various concentrations of it, simply by adding a twin fitting to their air intake to allow admission of a given proportion of 'clean' air to displace some of the open air. 'Clean' air was obtained from a compressed air

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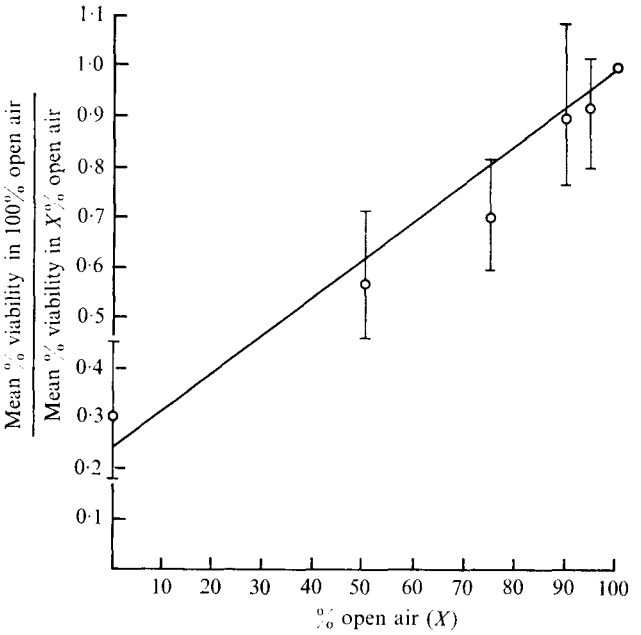


Fig. 5. Biological response of *Escherichia coli* to concentration of open air.

supply and suitably adjusted to ambient conditions of temperature and relative humidity to provide a metered supply to the sow. Such a supply was tested and found to be 'clean' in the sense that the viability of *E. coli* was not diminished when they were exposed to it for periods of 2 hr.

Using several sows it was possible to determine simultaneously the effect of various concentrations of 'open' air on viability. The results (Fig. 4) indicated that a 95 % concentration or less was significantly less harmful to the *E. coli* than 100 % open air.

At a given concentration of open air the variations in the mean viabilities were greater than those obtained in control experiments in the absence of OAF. These differences could be a reflexion of the day-to-day variation in the nature and concentration of OAF in open air in addition to the difficulties in maintaining constant ratios of open to clean air over the 1 hr. period of experiment. Nevertheless, a reasonably linear relationship was found between the ratio of mean viability and OAF concentration as shown in Fig. 5. When the biological response of *E. coli* in a ventilated system is similar to that observed concurrently in the open air then it may be concluded that similar concentrations of OAF are present.

## DISCUSSION

It was thought possible that the volume/surface area ratios of the ventilated vessels might correlate with the observed rate of loss of OAF. When the respective ratios were plotted against the maximum air dwell times (calculated from measured ventilation rates) for which OAF persisted in full concentration, a linear

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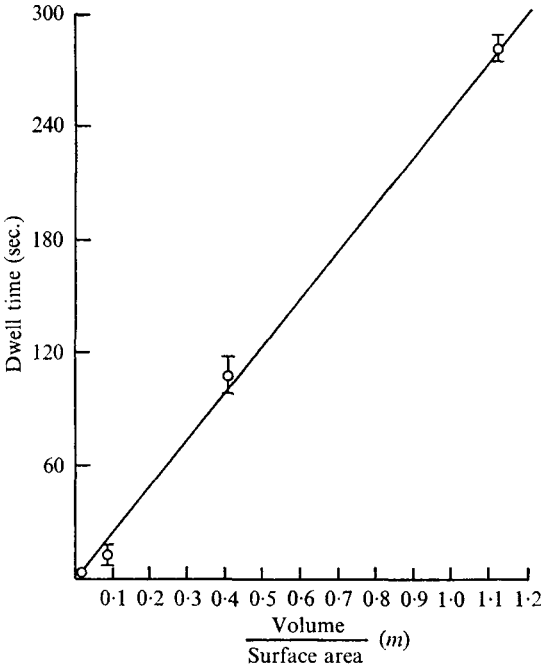


Fig. 6. Open-air factor (OAF) dwell time in vessels in relation to volume/surface area.

relationship was observed (Fig. 6). Furthermore, the curve produced showed an origin close to zero. The similar situation which obtains for the cuboid and cube is of special interest because although the cuboid is about twice the volume of the cube their volume/surface area ratios are similar. The results indicate a direct correlation of volume/surface area ratio with a rate of loss of OAF.

The effect of ambient wind on the air flow in some of the systems examined widened the limits of accuracy possible in this study. The data, however, indicate a near constant factor that can be used to calculate the maximum air dwell time for full preservation of OAF in enclosed systems. Multiplication of the volume/surface ratio ($m$) by 275 would give a close approximation to this time in seconds.

One explanation for the correlation obtained can be offered from Fick's first law of diffusion if it is assumed that loss of OAF in a vessel occurs by inactivation at the vessel wall. Rate of loss to walls is proportional to (i) the surface area of the wall, $A$, (ii) the diffusion coefficient for OAF, $D$, and (iii) the concentration gradient across the boundary layer, $c/b$, where $c$ is the concentration in the vessel used and $b$ is the thickness of the boundary layer. The total amount of OAF in a vessel of volume $V$ is $cV$, therefore the rate of loss, $d(cV)/dt$, is given by:

$$V\frac{dc}{dt} = -AD\frac{c}{b}. \tag{1}$$

On integration this gives $c = c_0 \exp\left[-\frac{DA}{bV}t\right]$,

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where $c_0$ is the concentration at zero time. Hence the characteristic time for OAF to be lost is proportional to $V/A$ provided that the thickness of the boundary layer, $b$, is constant. This is, in fact, approximately true since in fully developed turbulent conditions the thickness of the laminar boundary layer in a ventilated vessel is not very sensitive to the rate of dissipation of turbulent energy in the vessel (Landau & Lifshitz, 1959).

The experiments with OAF indicate that when the concentration is reduced by about 90% little bactericidal effect is apparent. To obtain this situation in the sphere a ventilation rate of approximately 5 air changes/hr. is required, thus indicating that 90% loss of OAF occurs in about 12 min.

Hence from equation (1):

$$\frac{D}{b}\frac{A}{V} = 3{\cdot}2 \times 10^{-3} \text{ sec.}^{-1}.$$

From Chamberlain's work (1967) with loss of $^{132}I$ vapour in large vessels $b$ can be calculated (since $D$ is known) to be 0·21 cm. The sphere $V/A$ is 112 cm. Hence $D$ for OAF is *ca.* 0·0752 cm.$^2$ sec.$^{-1}$.

From Graham's law,

$$M_1 = \frac{M_2 D_2^2}{D_1^2},$$

where $M$ is the molecular weight. Thus $M$ for OAF can be calculated by comparison, for example, with ethylbenzene ($D$ = 0·075 cm.$^2$ sec.$^{-1}$; Lugg, 1968) to be about 100 using the $D$ estimate for OAF. The accuracy of the experimental data obtainable is such that a molecular weight range of 50–150 is indicated. It is of interest that this would be within the molecular weight range of the ozone–olefin complexes previously suggested, on other grounds (Druett & May, 1969; Dark & Nash, 1970), as candidates for OAF. The molecular weight suggests that it is the initial complex of ozone and olefin that is bactericidal and not a breakdown product.

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