J. Hyg., Camb. (1971), **69**, 607
Printed in Great Britain

An indoor system for the study of biological aerosols in open air conditions

By A. M. HOOD

Microbiological Research Establishment, Porton Down, Salisbury, Wilts

(*Received* 7 *June* 1971)

SUMMARY

An indoor system designed for the study of survival of airborne micro-organisms in closed conditions has been successfully modified to allow the effect of open air to be measured. It was found that the unidentified open-air factors which are toxic for many species of microbes and rapidly lost when enclosed in conventional laboratory apparatus could be retained in the system by continuous ventilation at an adequate rate. The rate required allowed examination of *Escherichia coli* in aerosols generated from small amounts of material because of the short periods of ventilation required for appreciable viable decay to occur.

The validity of the system was tested by comparing the survival of *E. coli* in true aerosols with its survival when the droplets were held on microthread. An investigation of the role of relative humidity in open-air toxicity was included.

INTRODUCTION

The study of influence of open air on the viability of micro-organisms was facilitated by the development of the microthread technique for exposing them in a simulated airborne state (May & Druett, 1968). Unidentified pollutants in rural air have been described which are adverse to microbial survival and further characterized by their rapid removal from air when it is enclosed (Druett & May, 1968). Such ephemeral air pollutants cannot be demonstrated in conventional laboratory apparatus and the obvious hazards prevent a study of their effect on survival of aerosols of pathogenic micro-organisms in the field. A new technique is clearly required. The present investigation was made to determine whether an enclosed system could be ventilated in a way that would preserve these air pollutants for a sufficient time for their bactericidal effect to be manifest. If this could be achieved the study of survival and respiratory infectivity characteristics in open air of aerosols of pathogenic micro-organisms would be considerably enhanced.

MATERIALS AND METHODS

A system normally used for examination of aerosols held under hermetically sealed conditions was used. It consists essentially of a 22 ft. diameter mild-steel sphere (vol. $1{\cdot}67 \times 10^5$ l.) with a by-pass tube (side-arm) from which aerosol samples can be withdrawn and animals exposed (Fig. 1). An orifice of 1 ft. in

diameter in the top of the sphere is exposed to allow access of spray devices for aerosol dissemination. Air mixing inside the sphere is achieved by three fans situated near the base and when required air from the sphere is drawn through the side-arm by a fan at one end of the arm. The sphere is housed in a brick chamber with a concrete floor and the side-arm passes through one wall to an adjoining chamber of similar construction. The sphere chamber has access doors at the top and bottom and four extract fans in its roof to assist natural ventilation. These fans give a theoretical air change rate in the sphere chamber of about 20 air changes/hr.

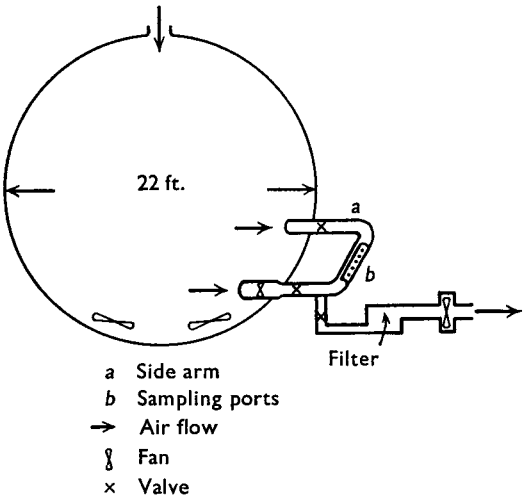

Fig. 1. Sphere system.

Modifications were made to this system to enable the sphere itself to be ventilated at rates of up to a maximum of 28 air changes/hr. Air is removed from the sphere via a T junction added to the side-arm, using a constant-speed extractor fan behind a triple bank of absolute filters, and replaced by air from the ventilated sphere chamber via the orifice in the top of the sphere. Ventilation rate was controlled by varying the restriction of the sphere air intake with knife-edged orifices of various diameters. Flow rate measurements were made with a Velometer (AEI Ltd) at the air intake. During these measurements a 1 ft. diameter tube 6 ft. long was attached to the intake to provide a laminar air flow.

E. coli MRE 162 (EC) was used to indicate, according to its viable decay rate, the toxicity of the air to which it was exposed. Spores of *B. subtilis* var. *niger* (BG) which are unaffected by the atmosphere were mixed with the EC to measure physical decay. Viable counts were made and EC viability assessed as described by May & Druett (1968). Aerosol samples were collected in multistage samplers (May, 1966) containing the collecting fluid described (May & Druett, 1968). In ventilated aerosol tests samples could be collected in still air from the side-arm by using a valve to by-pass the sampling ports without interruption of aerosol ventilation in the sphere. This was essential when efficient collection of large aerosol particles was required.

For aerosol tests about 3×10^{11} EC plus 1×10^{11} BG were sprayed directly into the centre of the sphere. When ventilated, loss of aerosol concentration due to physical removal proceeded at an exponential rate – about 90% loss in concentration every 10–15 min. Air samples of 500 l. were therefore required after 1 hr. ventilation in order to collect an adequate number of cells for assay. Two types of spray were used for dissemination of aerosols: a Collison spray with 18 jets which discharged about 1 ml. of fluid/min. when operated at 26 lb./in.^{2} air pressure and a two-fluid atomizer of the scent-spray principle operated at 80–100 lb./in.^{2} to discharge about 1 ml. fluid/sec. The former was used for production of small particulate aerosols of predominantly 1–2 μm. diameter and the latter for production of aerosols of much wider range of particle size.

In tests using the ventilated system, ventilation was started about 30 min. before exposure of micro-organisms. This established conditions of temperature and relative humidity very similar to those outside the building. Ventilation was continued without interruption until the end of the test. The high rates of ventilation used ensured that if a slight temperature differential existed between inside and outside the building the relative humidity was not affected by more than a few per cent.

Microthreads were loaded with particles containing EC/BG as described by May & Druett (1968). When they were exposed to open air during daylight hours they were housed in a roundabout (Druett & May, 1969) which shaded them from direct sunlight without preventing free access of air to them. When exposed to air in the sphere the frames were held in a wooden base by their handles, lowered to within a few feet of the bottom of the sphere and withdrawn at appropriate intervals during ventilation. Alternatively, they were inserted into exposure ports in the sphere side-arm. To avoid the adverse effect of air velocity on EC held on microthreads care was taken when possible to expose them in positions where the velocity would not be expected to influence survival (May & Druett, 1968). When this could not be done the microthreads were exposed simultaneously to similar air velocities in various parts of the system.

RESULTS

Introduction of toxic air to the system

All tests unless stated otherwise were made within the 70–95% range of relative humidity and between 3° and 20° C. Under such conditions the EC did not lose viability significantly during the exposure period in the non-ventilated system. The effect of ventilation was measured by comparing EC survival on microthreads held simultaneously inside the sphere and outside in open air. The results obtained at various ventilation rates indicated that (*a*) with 8 air changes/hr. no toxic air factors were present in the sphere, (*b*) with 9 air changes a proportion were present, and (*c*) with 12–20 air changes all toxic factors were apparently present since with these rates there was no difference between viable decay rate of EC inside and outside the sphere. Typical results are shown in Figs. 2 and 3.

The varying degree of toxicity of air from day to day was apparently associated

with the amount or nature of these air pollutants in the local air stream prevailing at the time of the test. From the results obtained it was concluded that a ventilation rate of over 12 air changes/hr. in the sphere was sufficient to maintain these pollutants at the same effective level as that found in open air in the locality.

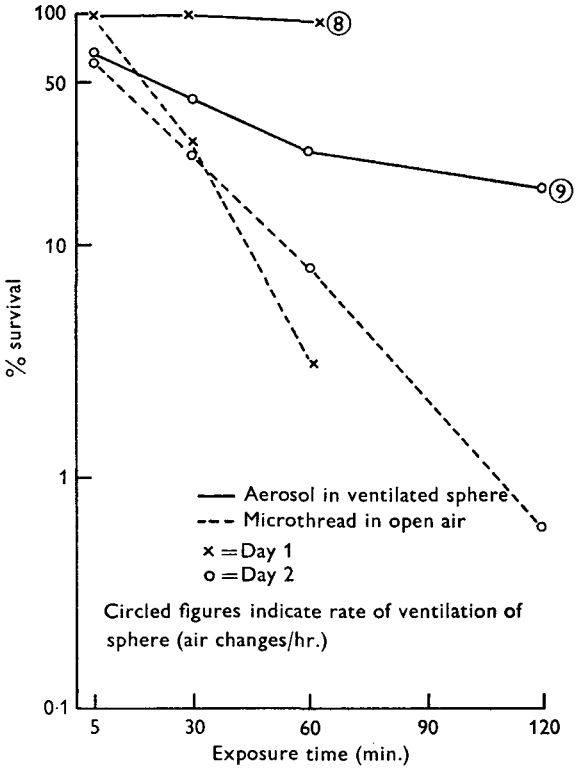

Fig. 2. *E. coli* survival on microthread in open air and simultaneously in aerosols in sphere ventilated at 8 and 9 air changes/hr.

Loss of toxic factors on enclosure

Loss of toxic factors in the air inside the sphere was very rapid when ventilation ceased. No further loss of EC viability in aerosols was observed when measured during subsequent periods of at least 20 min. (Table 1).

Ventilated aerosols and microthread exposure

Survival of EC in airborne particles $\leqslant 5\ \mu$m. diameter (i.e. those collected in the bottom stage of the multistage sampler) in the adequately ventilated sphere was compared with that in similar sized particles held simultaneously on microthread in open air. The results (Fig. 4 and Table 2) showed a slight difference – just significant ($P = 4{\cdot}8\%$) – between survival at 1 hr. in true aerosol and on microthread. The EC apparently survived slightly better on microthread than in the true airborne state.

https://doi.org/10.1017/S0022172400021884 Published online by Cambridge University Press

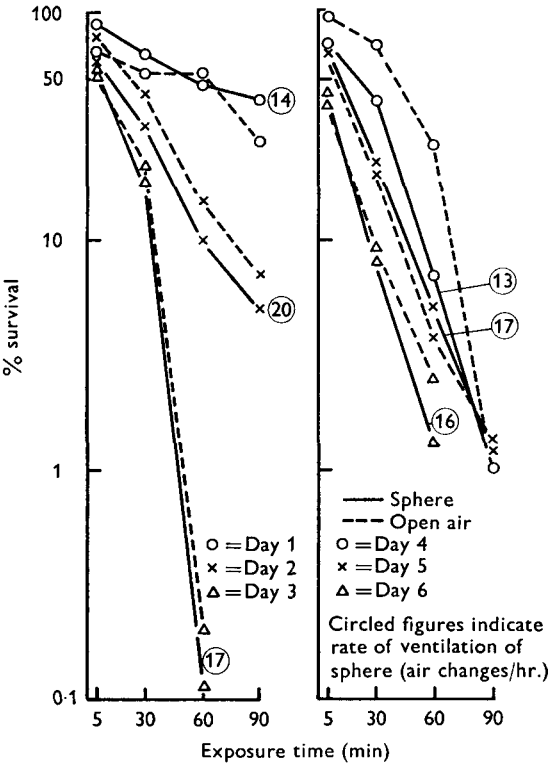

Fig. 3. *E. coli* survival on microthread in open air and simultaneously in sphere ventilated at rates between 12 and 20 air changes/hr.

Table 1. Escherichia coli *survival* (%) *in aerosols of* ⩽ 5 *μ particles: effect of ventilation for* 45 *min. followed by closed conditions* 45–65 *min.*

Aerosol age (min.)	Ventilated			Non-ventilated		
	5	30	45	55	60	65
Test (day 1)	51	49	6	7	7	7
Test (day 2)	63	37	2	2	1	3

Particle size response to toxic air

The results of exposing EC in aerosols and on microthread to solar radiation indicated that survival was poorer in small particles than in large ones (May & Druett, 1968). The practical difficulties limited those aerosol tests to airborne holding times of about 3·5 min. These are overcome by using the present system in which aerosols can be held for long periods. Tests for the effect of open air on true aerosols in the absence of sunlight could thus be made in the sphere at any time of day. The purpose was to determine whether the open air toxic factors exhibited a particle size effect on EC survival in the absence of sunlight.

Aerosols of wide range of particle size were ventilated and samples removed at intervals during their 1 hr. period of ageing. The three particle-size fractions

https://doi.org/10.1017/S0022172400021884 Published online by Cambridge University Press

(< 3, 3–6 and > 6 μm. diameter) obtained with the multistage sampler were assessed for EC viability and decay curves plotted. The results showed that the effect of toxic air on EC survival was almost invariably associated with a particle-size effect, viable decay proceeding more rapidly as particle size diminished. The difference between the viable decay rates of the three particle-size fractions was

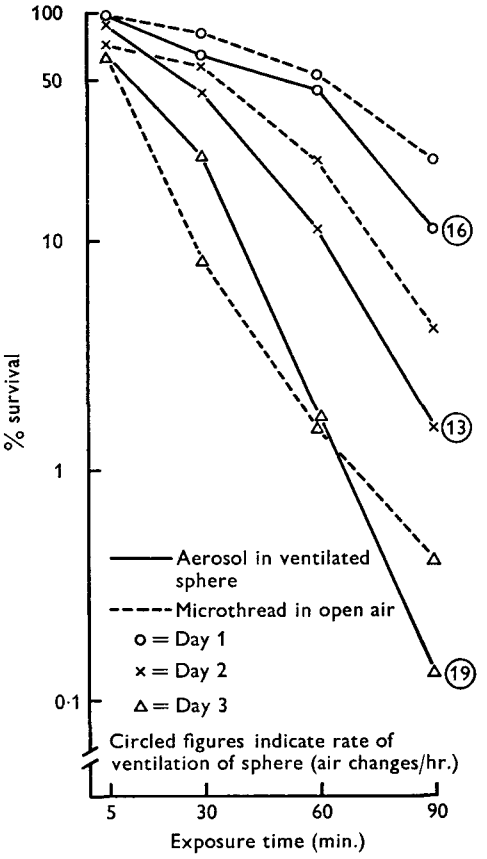

Fig. 4. *E. coli* survival on microthread in open air and simultaneously in aerosols in ventilated sphere.

not constant from one test to another even when relative humidity and temperature conditions were similar. Some examples of the types of response obtained are shown in Fig. 5. The results confirm the conclusions reached by Druett & May (1968) from their work with microthread, that the nature and concentration of the unstable germicidal pollutants in rural air are extremely variable and not associated with time of day or season.

Toxic air factors and relative humidity

Druett & May (1968) suggested that rapid fluctuation of relative humidity such as occurs in open air might contribute to the adverse effect it has on microbial

https://doi.org/10.1017/S0022172400021884 Published online by Cambridge University Press

survival. They failed to demonstrate any effect of artificial fluctuation in laboratory air.

During the present series of tests an instrument was used to record rapid changes in wet-bulb depression (W. C. Wright, personal communication). It was found that the rapid changes in relative humidity occurring in open air were not reflected inside the sphere (Fig. 6). Measurements made in several positions in the ventilated

Table 2. Escherichia coli *survival in ventilated aerosols and on microthread held simultaneously in open air*

Aerosol age (microthread exposure time)			
5 min		60 min	
% viability			
(*a*)	(*b*)	(*a*)	(*b*)
74	68	9	3
51	69	6	12
84	89	16	42
86	85	10	20
90	89	12	14
75	80	5	14
87	88	4	27
78	57	6	2
68	43	3	2
45	69	1	3
78	57	6	2
46	57	4	27
71	77	2	4
80	61	4	8
70	52	12	22
Average values			
72	69	7	13

(*a*) Aerosol particles collected in bottom stage of multistage sampler from ventilated aerosols in sphere.

(*b*) Particles held on microthread in open air.

sphere, including one within a few feet of the air intake, gave similar answers. Since the EC exposed on microthread survived similarly under both conditions of relative humidity, i.e. in the ventilated sphere and in open air, it was concluded that rapid changes in relative humidity had no apparent effect on the survival of EC.

Survival curves of EC in ventilated and non-ventilated aerosols were obtained at various relative humidities within the 40–95% range as indicated by conventional wet and dry bulb thermometer readings. These were compared to determine whether any correlation existed between the effect of open air factors and relative humidity. The results (Fig. 7) showed that in the absence (non-ventilated) of open air factors EC survival was not significantly affected at relative humidities of 71–95% but was adversely affected at lower relative humidities. It was found that the adverse effect of 'open air' could be large irrespective of the relative humidity.

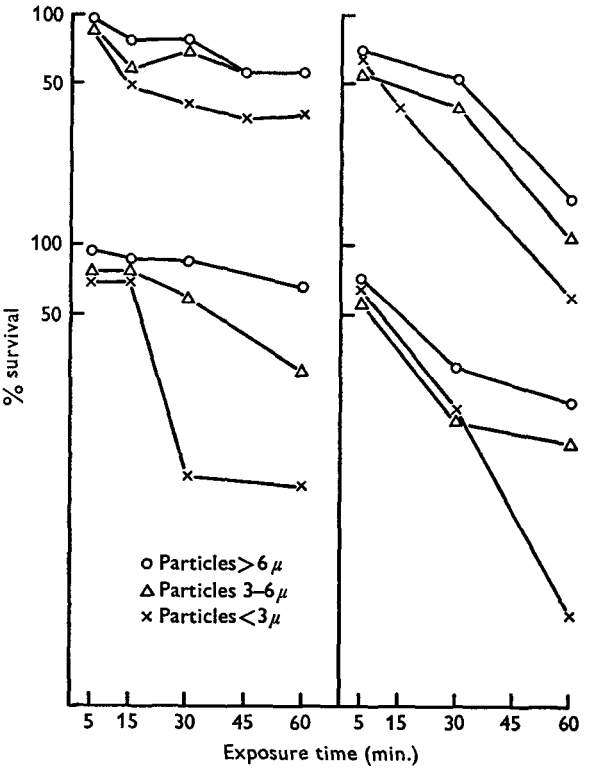

Fig. 5. *E. coli* survival in aerosols ventilated in spheres: effect of particle size.

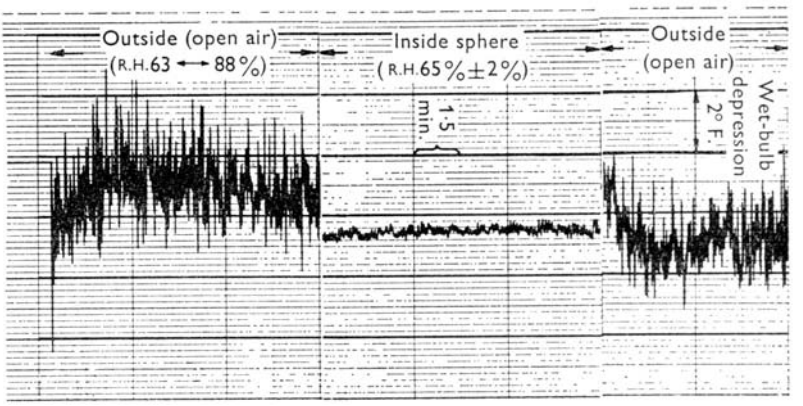

Fig. 6. R.H. fluctuation inside sphere and outside (open air).

Tests with air-intake tube

The possibility of using ducted air for ventilation of the sphere was examined because it afforded the distinct advantage of a controlled air intake pathway. The work of Druett & May (1968) indicated that some loss of toxic factors occurred when open air was passed through a 4·5 in. diameter tube. It was important therefore in a system designed to retain and measure the effect of unmodified open air to examine the effect of any duct applied to the air intake of the system.

https://doi.org/10.1017/S0022172400021884 Published online by Cambridge University Press

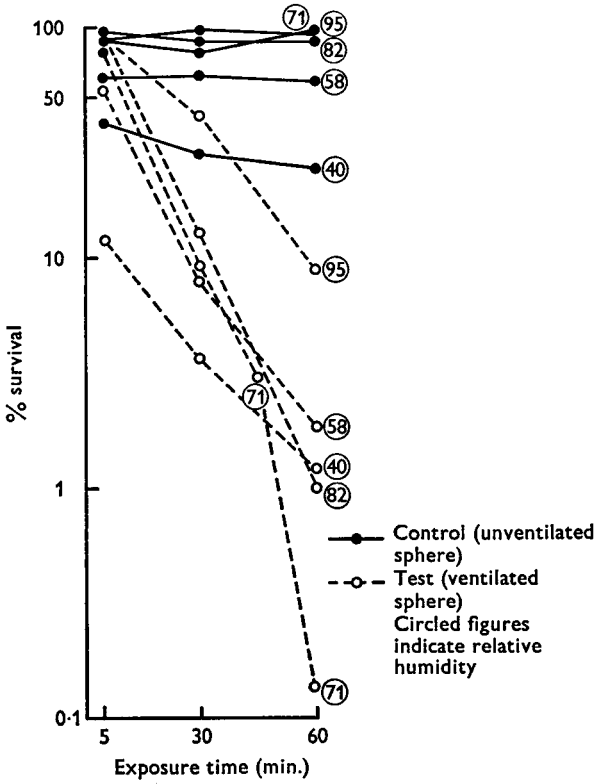

Fig. 7. *E. coli* survival in aerosols: effect of open air and relative humidity.

An air-intake tube of stainless steel 15 ft. long and 1 ft. diameter was used. This was of similar cross-section to the sphere side-arm and thus air velocity in both intake and extract situations would be similar at any given ventilation rate, thereby overcoming some of the difficulty in interpreting results associated with the adverse effect of air velocity on EC survival when held on microthread (May & Druett, 1968). The intake tube was connected from outside, through the sphere chamber wall to a stainless-steel box (*ca.* $3 \times 2 \times 2$ ft.) which formed a connecting piece between the tube and the sphere intake orifice. The steel box was fitted with a safety-glass panel, gloves and an airlock to afford access to the inner end of the tube and to the inside of the sphere.

Viable decay curves of EC exposed on microthread were used to measure the degree of air toxicity as it passed through the system. Various rates of ventilation were used. It was found that although there was no apparent loss of toxicity in the intake tube at linear air speeds as low as 6 ft./sec. some loss occurred between this tube and the extract tube (sphere side-arm). In order to obtain air in the latter of similar toxicity to that in the intake tube an air speed in excess of 25 ft./sec. was required. This rate of ventilation corresponded to that which gave about 12 air changes/hr. in the sphere. The results indicated that the loss of toxic factors from ducted air – presumably due to wall losses – is slower in 1 ft. diameter tube than in the 22 ft. diameter sphere, and confirmed that in this system it is the residence

time of the air in the sphere that governs the minimum ventilation rate required to maintain the toxic factors in full throughout the system.

Further tests were made using the modified system in which EC survival in ventilated aerosols was compared with survival on microthread in open air. The results obtained at various rates of ventilation were so similar to those found previously without an air-intake tube that it was concluded that any effect of the intake tube could be disregarded as far as loss of toxicity was concerned.

DISCUSSION

The presence and activity of factors in open air toxic for EC has now been demonstrated in an indoor system. Loss of toxicity occurs quickly when air is contained and hence a high ventilation rate with fresh air is required to maintain its effectiveness. Since the loss is apparently due to removal by surfaces the size and geometry of the chamber influences the rate of ventilation required. The minimum rate indicated for the system examined is about 12 air changes/hr. for full preservation of open-air toxicity. The results obtained at various rates of ventilation suggest that the half-life of the toxic factors is about 3 min. in this particular system. This is of similar order to that found by Arnold (1959) for the phytotoxicant product from a 2-pentene-ozone reaction in a tube apparatus. This product has also been shown to be active against EC viability (Dark & Nash, 1970). The present results therefore support the indication that the as yet unidentified bactericidal pollutants in open air may be the reaction products of natural ozone and gaseous petroleum products from innumerable sources.

The most important outcome of this investigation is the discovery that the technique necessary to produce a 'natural' air condition in the sphere system examined enables aerosol tests to be made simply and with small amounts of material. Any large container is potentially suitable provided that the high removal rate of the airborne micro-organisms leaves sufficient material for effective assay over the operating period required. Since such an enclosed system removes any possible direct radiation effect of daylight the range of ambient conditions of temperature and humidity available for study is not restricted.

The results obtained strongly indicate that the ventilated system described provides a facility in which the effect of unidentified toxic factors in open air on microbial aerosols can be examined in detail. With proper safety precautions to protect laboratory personnel and thorough treatment of the air extracted from the system by filtration and heat-sterilization a safe system can be provided for work with pathogenic materials. It will thus be possible to determine the effect of open air on aerosols of pathogenic micro-organisms with particular reference to the relationship between viability (as determined *in vitro*) and respiratory infectivity measured by animal exposure.

I wish to thank I. H. Silver for his interest and advice in preparing this paper, Mrs H. I. Willis, C. B. Morris and J. Ching for technical assistance and I. Myles of the Chemical Defence Establishment, Porton, for facilitating the engineering improvisions required.

REFERENCES

ARNOLD, W. A. (1959). The longevity of the phytotoxicant produced from gaseous ozone-olefin reactions. *International Journal of Air Pollution* **2**, 167.

DARK, F. A. & NASH, T. (1970). Comparative toxicity of various ozonized olefins to bacteria suspended in air. *Journal of Hygiene* **68**, 245.

DRUETT, H. A. & MAY, K. R. (1968). Unstable germicidal pollutant in rural air. *Nature, London* **220**, 395.

DRUETT, H. A. & MAY, K. R. (1969). The open air factor. *New Scientist* **41**, 579.

MAY, K. R. (1966). Multistage liquid impinger. *Bacteriological Reviews* **30**, 559.

MAY, K. R. & DRUETT, H. A. (1968). A microthread technique for studying the viability of microbes in a simulated airborne state. *Journal of General Microbiology* **51**, 353.