AN APPARATUS FOR THE STUDY OF AIRBORNE INFECTION

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(With 5 Figures in the Text)

INTRODUCTION

The apparatus described in this paper was designed some years ago to study the survival of bacteria suspended in the atmosphere as dry particulate clouds, and the effect of such clouds of pathogenic bacteria when inhaled by experimental animals.

Several different workers have used this apparatus since its first introduction, so that the experience on which the following account is based is the collective experience of those members of the staff of M.R.D. who have been at different times concerned. The apparatus has been repeatedly referred to in publications, always with the promise that a report on design and working would follow. The following account is intended to supplement such references, and to meet requests for information on the subject.

Rosebury (1947) has reviewed the literature on laboratory apparatus designed for the study of airborne infection. All kinds have their limitations, ranging from over-complexity in design and use to inefficient operation either in the method of producing the bacterial cloud or in estimating the cloud concentration.

The advantages of this apparatus are:

(*a*) Relative simplicity of design and operation.

(*b*) Regularity with which any desired cloud concentration can be established and assessed.

(*c*) Constancy of results with experimental animals.

(*d*) No worker has contracted infection as a result of handling the apparatus according to instructions. It is, therefore, safe to use within the limits reasonably required by the research worker.

Disadvantages in its use are:

(*a*) It can produce only clouds of single organisms.

(*b*) The method of producing the cloud from liquid suspension in certain circumstances damages sensitive organisms.

(*c*) An occasional organism may escape. Under proper conditions, however, the numbers that do so are not detectable by the most efficient air-sampling devices. The evidence for leakage is based solely on post-operative safety checks by swabbing benches, etc., after spore-forming organisms have been used in an experiment.

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(*d*) Bearing item (*c*) in mind the use of gowns, gloves and respirators is advisable during the course of an experiment.

THE CIRCUIT

The apparatus produces a fine 'dry' particulate cloud of bacteria from a suspension in some suitable liquid in such a manner that:

(*a*) Samples of the cloud may be conveniently collected for the estimation of viable organisms.

(*b*) Animals may be exposed for the purpose of inhaling the cloud for varying periods of time.

(*c*) 'Waste' cloud may be filtered so that the effluent air is innocuous.

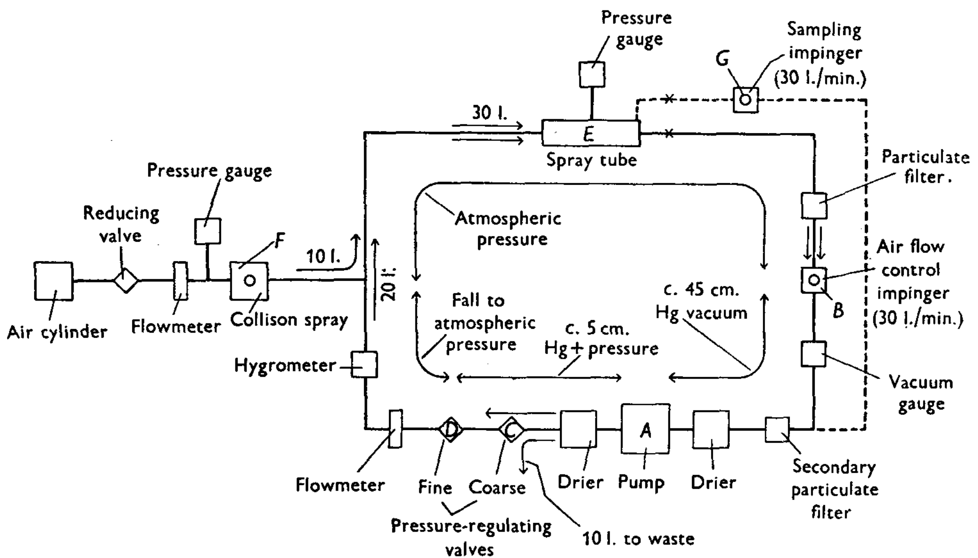

Fig. 1.

It works as a closed system from the point of spray output until after the 'used' cloud has been efficiently cleared of organisms.

Fig. 1 shows diagrammatically the flow throughout the system. The principal centres of control are the pump, *A*, the critical orifice controlling the air-flow, *B*, and the pressure regulating valves, *C* and *D*. The pump must be of such capacity that the critical orifice operates at maximum flow rate. For practical purposes this is achieved if the pressure difference across the orifice is half an atmosphere or greater; thereby a constant flow of air is maintained. The pressure in the animal exposure tube, *E*, is controlled by the simple pressure regulating valves, *C* and *D*. Air coming from the pump passes through these valves and the pressure is there adjusted so that under normal working conditions the air in the animal exposure tube is being pulled toward the pump at a mild negative pressure of about 0·5 cm. Hg.

The bacterial cloud is introduced to the system by means of the spray, *F*, operated by compressed air at a constant pressure. The air emerging from the

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regulating valves, C and D, mixes with the air from the spray system, and the diluted bacterial cloud passes through the animal exposure tube and thence through the critical orifice, B, on its way back to the vacuum pump. The cloud passes through two particulate filters of high efficiency (see below, p. 59), one placed immediately in front of the critical orifice and the other immediately behind. The filtered air then passes through two silica gel driers which control humidity, one placed immediately before and the other immediately after the pump. Thereafter the air passes once more through the pressure regulating valves in transit to the animal exposure tube, meeting in its path fresh bacterial cloud. The process is continuous.

An important by-pass system is incorporated for sampling the cloud in such a way that accurately known amounts are collected for *in vitro* assay or for animal tests by parenteral injection. This is achieved by drawing the cloud off at point G at the distal end of the animal exposure tube. It is passed through one or two forms of high efficiency collecting devices (see below), and the effluent air therefrom by-passes the main critical orifice and enters into the circuit again immediately before the second particulate filter. The flow from the collecting device is controlled by means of a critical orifice carefully balanced with that in the main circuit. This latter is closed while the by-pass is open and the pressure balance within the system is thereby maintained.

NOTES ON SPECIAL PARTS OF THE APPARATUS

Working drawings of the various parts that are not standard pieces of equipment are available. The descriptions that follow, therefore, are accompanied only by simple sketches.

(1) *The spraying apparatus and the cloud produced*

A modified pattern of the original Collison atomizer has been used. Fig. 2 shows the spray held in position in a glass container by means of a metal screw top. The principle of operation is that compressed air passing down the tube A emerges at high velocity at point B on the spray head. Liquid is thereby sucked up the tube C in the spray head, emerging with the air by which it is shattered. The coarser droplets are impinged on the sides of the glass container; the finer ones are carried up with the stream of air to the exit point on the spray bottle. Normally about 150 ml. are placed in the bottle which has an internal diameter of about 6 cm.; the height of the point of exit of the spray above the liquid is approximately 10 cm. The spray is operated under 26–27 lb. air pressure and the rate of flow, which varies slightly with each spray head, is of the order of 10 l./min. Continuous spraying of aqueous solutions or suspensions leads to a concentration of the material as a result of the removal of water vapour. It is estimated that in a spray of this pattern the amount of water lost as vapour is approximately equal to the calculated water droplet output. By using 150 ml. of liquid the loss of water as vapour after 30 min. spraying is probably not greater than 1–2 %.

The range of particle size produced by the spray may be considered as: (*a*) the droplet size as it emerges from the mouth of the spray bottle, and (*b*) the particle

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size of any substance in solution or suspension after extraneous water has been evaporated. The range of dried particle size produced by spraying 1 % methylene blue under the conditions specified above has been measured, and is given as:

μ	%	μ	%
0·2	62·1	1·2	1·6
0·4	28·3	2	0·2
0·8	7·8		

It appears, therefore, that about 90 % of the droplets as they emerge from the spray are less than 10 μ in diameter. The concentration of bacterial suspension used is never greater than 1×10^9. This dilution is such that very few droplets can contain more than one organism, and sedimentation studies of clouds produced from such suspensions show that about 95 % of dried particles consist of single organisms.

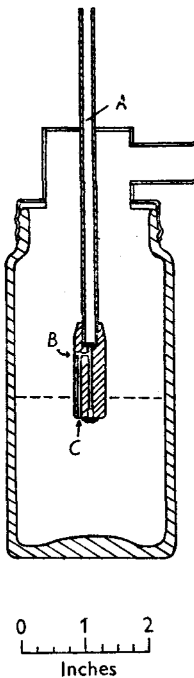

Fig. 2.

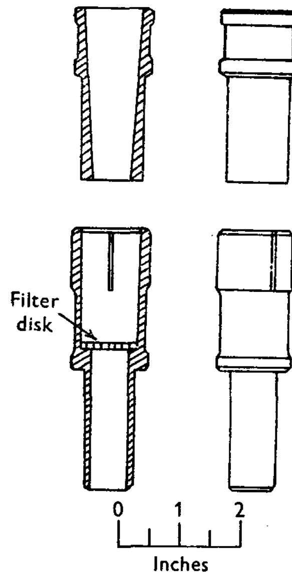

Fig. 3.

(2) *Methods for cloud sampling*

(*a*) *The liquid impinger*

This method was first brought to my notice by Prof. Gaddum. It consists of a modification of the midget impinger described by Littlefield, Feicht & Schrenk (1937), and has recently been described again by Rosebury (1947) for use in the collection of bacterial clouds.

(*b*) *The microfilter*

This useful device was designed by Dr Toms, at one time at C.D.E.E., Porton. Fig. 3 shows the general design. Disks of filter material are cut by cork borer exactly to fit the filter holder. The material used is generally merino wool either

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mixed with asbestos, resin coated, or treated with gum acacia. The amount of filter material to be used is adjusted according to the degree of resistance to flow permissible in any experiment. Generally this is of the order of 26 cm. water gauge in a flow of 10 l./min. Cloud-volume control is effected by the use of a critical orifice operating on the output side of the filter. After collection the filter pads are placed in a suitable liquid, the whole shaken, and dilutions made for bio-assay.

Representative experiments designed to test the relative merits of these two methods of cloud collection are given later in the text.

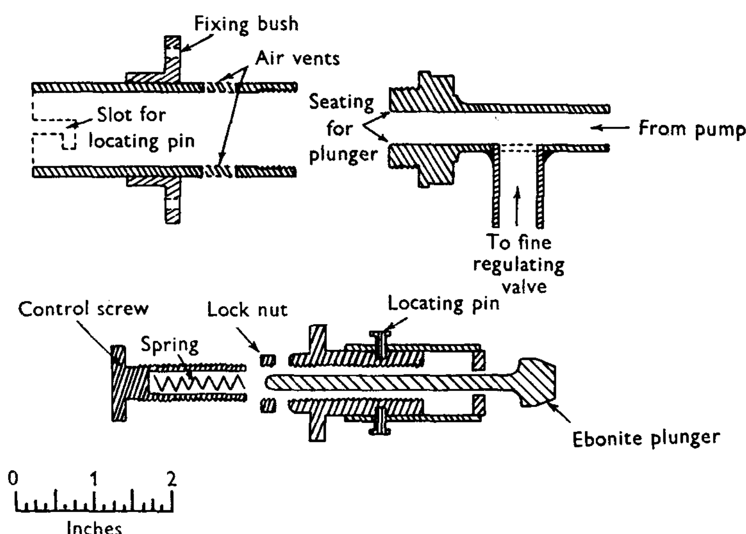

Fig. 4.

(3) *The vacuum pump*

This is a rotary pump with an air displacement of 224 l./min. (Messrs Edwards, London) which can maintain a vacuum of 40–50 cm. Hg on the discharge side of an impinger, with a total air-flow up to about 40–50 l./min. Such a vacuum is sufficient to maintain the maximum rate of flow. By this means the collecting efficiency is at its optimum, and the precise volume of cloud collected in a given period of time is known.

(4) *Air-flow control*

A piece of glass capillary tubing of the right bore is used to give the maximum flow rate desired, which is never greater than 50 l./min. at S.P.T.

(5) *The pressure regulating valves*

Fig. 4 shows the design of one of these simple valves. When they are fully open, air flowing from the pump freely passes either back into the circuit, or to the exterior atmosphere. The proportion of the total flow passing to the former is determined by the difference in pressure within the circuit and that of the external atmosphere. When the Collison spray is not in action the volume of the air

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intake to the circuit is exactly equal to that removed through the air-flow control impinger. With the Collison spray operating, and at a constant rate of flow, the air intake is then equal to that removed through the air-flow control impinger less the input of the spray. The resistance to flow created by tubing, connexions, and the flow meter between the pressure valves and the spray tube is such that, with the valves fully opened, a vacuum of about 4 cm. Hg can be maintained within the spray tube. When the spray is functioning, this vacuum is reduced to about 2 cm. Hg. In circumstances where the circuit is entirely sealed from the point of air input, there is in fact no necessity to put the valve into operation. If, however, the circuit is broken (as, for example, at the distal end of the spray tube when animals are exposed) then not more than 0·2 cm. Hg vacuum is permissible because the rubber seal around the animal's nose forms only an imperfect vacuum seal. Attempts to expose animals with the pressure regulating valve open therefore result in (*a*) local cloud turbulence and irregular concentration around the animal's nose, and (*b*) the possibility of imperfectly dried and mixed clouds due to the reduction in the amount of dried air entering with the spray. This difficulty is entirely overcome by regulating the pressure valve in such a manner that a positive pressure of a few cm. Hg (5–10) is maintained between it and a fine adjustment valve. By regulation of this second valve the desired pressure can be maintained within the spray tube.

(6) *Animal exposure tube*

This is of metal, about 2 in. in diameter, and has an effective length of about 26 in. At the distal end there are two exposure points for guinea-pigs or for rabbits.

(7) *Animal exposure points*

An important feature of the apparatus is that arrangement is made for the nose and mouth only of the experimental animal to be exposed to the passing cloud. This is done by one of a variety of methods, but basically the nose and mouth of the animal are inserted through a hole in a thin rubber diaphragm. For the exposure of guinea-pigs and rabbits, exposure points are built into the spray tube at its distal end. As noted above, when used properly the seal obtained is sufficient to maintain about 0·2 cm. Hg vacuum within the exposure tube. For larger animals such as monkeys and sheep, a rubber hose connexion is fixed to the end of the animal exposure tube, and a rubber diaphragm attachment fitted similarly at the end of the hose. This procedure is essential because of the physical disturbance created by these larger animals. Care is taken to reduce the amount of 'dead space' between the nose of the animal and the passing cloud. Whichever method is in operation the estimation of cloud concentration is made by collecting representative samples at points immediately adjacent to those used for exposing the animals. Animals are suitably held in boxes or crates in which the head is held by a sealed neck attachment reminiscent of the stocks.

Experience has shown that two is the optimum number of guinea-pigs to attempt to expose for a period of about 1 min. to passing clouds. For similar

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exposure time, one rabbit, monkey, or sheep is the optimum number. Mice do not appear to be suitable for such cloud studies, and a description of methods for exposure of these animals is deferred.

(8) *Control of humidity*

It is essential that the air entering the spray tube should be of sufficiently low humidity to ensure that the cloud particles in their transit along the tube will lose all the water surrounding them. The Physics Section, Porton, has supplied the data in Table 1 concerning the time taken for the evaporation of water droplets of different sizes at 15° C. in atmospheres of different humidities.

Table 1. *Evaporation of water droplets*

Droplet diameter (μ)	Percentage relative humidity 0	25	50	75
	Life in air (sec.)			
1	$0\cdot29\times10^{-4}$	$0\cdot39\times10^{-4}$	$0\cdot58\times10^{-4}$	$1\cdot18\times10^{-4}$
2	$1\cdot16\times10^{-4}$	$1\cdot55\times10^{-4}$	$2\cdot3\times10^{-4}$	$4\cdot7\times10^{-4}$
5	$7\cdot3\times10^{-4}$	$9\cdot7\times10^{-4}$	$1\cdot5\times10^{-3}$	$2\cdot9\times10^{-3}$
10	$2\cdot9\times10^{-3}$	$3\cdot9\times10^{-3}$	$5\cdot8\times10^{-3}$	$1\cdot2\times10^{-2}$
20	$1\cdot16\times10^{-2}$	$1\cdot6\times10^{-2}$	$2\cdot4\times10^{-2}$	$4\cdot7\times10^{-2}$
90	0·25 sec.	0·3 sec.	0·5 sec.	1 sec.

The dimensions of the animal exposure tube noted above are such that with a rate of flow around 30 l./min. approximately 2·5 sec. is taken in transit along the tube. About 90 % of droplets coming from the spray are less than 10 μ in diameter. It is clear, therefore, that even at 75 % relative humidity at the point of exit from the spray tube there is a wide safety margin. Normally a standard of 50–60 % humidity is maintained by the use of silica gel driers. Before starting an experiment water is sprayed and the humidity checked by wet- and dry-bulb thermometers that are fixed temporarily in the circuit.

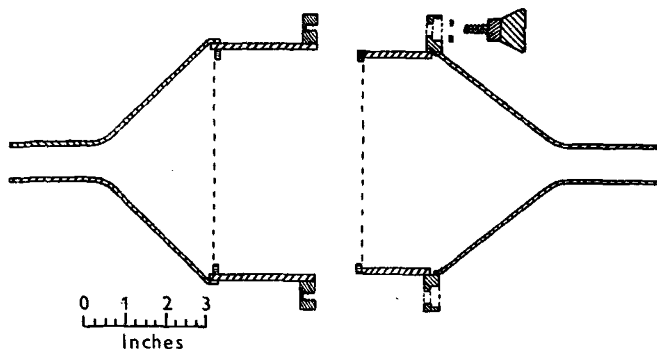

Fig. 5.

(9) *Filters for removing particulate matter from waste cloud*

A sketch of the filter jig is given in Fig. 5. The filtering material is merino wool impregnated with resin, and has a penetration for particles of about 0·5 μ of the

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order of 1×10^{-7}%. In view of the fact that at least two of these filters are used in series, it is clear that the chances of organisms escaping by this route are so low as to be an acceptable risk.

EXPERIMENTAL PROCEDURE

(1) *Precautions*

The worker wears a gas mask with a specially fitted particulate filter similar to that used in service respirators but excluding the carbon charging. A hood covering the head and shoulders, as well as a gown and surgical gloves, are also worn. These precautions might be regarded as excessive, but laboratory accidents due to airborne infection are of such frequent occurrence that the discretion is advisable.

A useful accompaniment to these procedures when vegetative pathogens are being used is the generation of a chemical disinfectant aerosol such as hexyl resorcinol in the operating room.

As considered necessary, air samples from the spray room are taken with a Bourdillon slit sampler (1941) if the organisms being sprayed will grow on media which can be used in it. After an experiment, the apparatus is broken down into the smallest number of sections convenient for autoclaving.

(2) *Details of working procedure*

(*a*) The only parts of the apparatus which must be sterile before use are the cloud sampling devices.

(*b*) The flow through the Collison spray at the working air pressure is checked against the flowmeter. For the purpose of calibration, water replaces the toxic suspension in the Collison spray bottle.

(*c*) The apparatus is then assembled.

(*d*) Both pressure-regulating valves are fully opened.

(*e*) The vacuum pump is switched on.

(*f*) The Collison spray is put in action.

(*g*) The coarse pressure-regulating valve is brought into action and the associated manometer adjusted to about 10 cm. Hg positive pressure.

(*h*) The fine adjustment pressure gauge is then operated until the pressure registered in the spray tube is atmospheric (or about 0·2 cm. Hg negative pressure).

(*i*) A reading on the air input flowmeter is taken.

(*j*) The animal exposure point along the spray tube is opened and another reading as at (*i*) above is taken.

(*k*) By slight adjustment of the fine adjustment pressure gauge, a position is reached when the flowmeter reading does not alter when the animal exposure point is either open or sealed. In other words, the air input from the pump exactly cancels the air intake required by the apparatus. For reasons given above, this is of considerable importance when animals are being exposed.

(*l*) A reading is taken on the vacuum gauge associated with the output side of the airflow control impinger. Operating conditions require not less than 41–54 cm. Hg.

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(*m*) A vacuum gauge should also be connected immediately after the particulate filter. Resistance to flow through the filter produces a vacuum of about 1–2 cm. Hg. It should be observed that the effective vacuum on the output side of the control impinger is in fact the reading there given less the vacuum registered before entering the impinger.

(*n*) The Collison spray is turned off.

(*o*) The vacuum pump is switched off.

(*p*) The coarse pressure regulating valve is withdrawn by disconnecting from a locking pin position.

(*q*) Conditions are thus stabilized and the apparatus is ready for use.

(*r*) The toxic suspension replaces the water in the Collison spray bottle.

(*s*) An animal is placed in position.

(*t*) The vacuum pump is turned on.

(*u*) Within 2–5 sec. the coarse pressure regulating valve is re-engaged. This step is essential if momentary positive pressures are to be avoided in the spray tube due to the first push of air from the pump.

(*v*) The spray is turned on and the exposure is timed from the instant the proper working pressure is attained (about 2 sec.).

(*w*) The end of the time of exposure is judged from the time first action is taken to turn off the spray (about 2 sec.).

(*x*) Filtered air from the pump is allowed to flow through the apparatus for 15–30 sec.

(*y*) The vacuum pump is turned off, thus creating a moderate vacuum in the spray tube.

(*z*) The animal's nose is withdrawn in such a manner that a piece of wetted lint held below is swept up and over the nose.

(*aa*) *Cloud sampling.* The apparatus is worked as above, but with the animal exposure point sealed. When the mask for large animals is in use, a rubber bung is held in the opening of the mask diaphragm. When working conditions are stabilized, the flow through the air-flow control impinger is diverted through the sampling impinger by the instantaneous closing of one lead and the opening of the other (by means of artery forceps). At the end of the sampling period this process is reversed. Subsequent procedure is as above.

ESTIMATION OF ORGANISMS IN CLOUDS

(1) *Terminology of the assessment of cloud concentration*

(*a*) *The absolute spray factor* (A.S.F.)

In the processes of spraying, drying, and collecting of organisms there are losses due to trauma; there are also mechanical losses such as deposition of cloud particles during passage from the spray bottle to the animal exposure point, and during collection in, and recovery from, cloud sampling devices.

It is important to be able to differentiate losses due to trauma from those of mechanical origin. These latter losses can be assessed separately by spraying a suitable chemical substance in solution and estimating the amount collected

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per unit volume of cloud. The results so obtained are expressed in percentage as the ratio of the measured amount of substance per unit volume of cloud to the amount of substance per unit volume in the solution sprayed; this is called the absolute spray factor (A.S.F.).

In estimating the A.S.F. operating conditions must be kept constant, and a stable indicator substance must be used. The dye Sky Blue F.F. (colour index no. 518) has been found satisfactory for this purpose. If it is mixed either with a bacterial suspension similar to that to be used for an experiment, or with the experimental suspension itself, constant results are obtained provided the concentration of the bacterial suspension is not greater than about 10^8 per ml. and that of the dye about 1 % (see below).

(*b*) *The viable spray factor* (V.S.F.) *and the spray factor ratio* (S.F.R.)

When bacterial suspensions are used the spray factor is calculated in terms of viable organisms and described as the viable spray factor (V.S.F.). Usually, the V.S.F. differs from the A.S.F. Among other things the V.S.F. depends largely on the filtration and recovery efficiency of the sampling device, and on the sensitivity of the organism to spraying, drying and collecting. The ratio V.S.F./A.S.F. is defined as spray factor ratio (S.F.R.) and expressed as a percentage. It is useful in deducing causes of change in V.S.F.

There are other methods for estimating the efficiency with which a cloud has been produced and collected; for example, the most commonly employed principle is Rosebury's, in which the absolute amount of material sprayed, and the amount accounted for in cloud form are estimated. Ideally this is the method of choice, but in practice the method described above limits the unknown factors to filtration efficiency of, and recovery from, the sampling device. This opinion is strengthened by the evidence presented in the next section.

(2) *The efficiency of cloud sampling by impinger and by microfilter*

Two factors are involved, namely:

(*a*) The filtration efficiency, i.e. the percentage of particles actually taken out of the cloud passing through the sampling device.

(*b*) The recovery efficiency, i.e. the percentage of particles in the sampling devices which can be extracted and assayed by biological technique.

The overall efficiency is equal to the product of these two separate efficiencies. Differences detected between the two sampling devices, estimated by comparing the number of particles obtained from each device, can be attributed to a combination of the two factors. The filtration efficiency can, however, be measured separately by passing the effluent from a sampling device through one or more other samplers, provided always that by arrangement all samplers are working at maximum efficiency. In this section attention is confined to filtration efficiency. Recovery efficiency is discussed later.

The efficiency of an impinger is determined by:

(*a*) The velocity of air flow. This must be maximum, and as already noted is attained when the pressure drop across the orifice is at least half an atmosphere.

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(*b*) Under certain conditions the frothing of the liquid due to violent agitation may send a very fine spray over with the effluent air. In addition, if the collection is prolonged, an error arises due to evaporation. For large volumes, e.g. 50 ml., and short times of sampling, e.g. 1–2 min., the effect of evaporation is quite inappreciable.

The efficiency of the microfilter is determined by:

(*a*) The basic efficiency of the fine fibres. With the materials used, particles greater than 1 μ in diameter will be held in the interstices, and here the efficiency is independent of the velocity of the air flow. Particles down to 0·5 μ, or perhaps as small as 0·2 μ, will be held in the filter by impaction on the fibres, and efficiency depends here on the velocity of air flow. Finally, some very small particles will be in Brownian movement and eventually get impacted on the fibres.

(*b*) Construction of the microfilter. Crude leaks around the edges of the pad of fibre may occur unless special precautions are taken. Table 2 shows the results of a series of paired comparisons of filtration efficiency between impingers and microfilters. The cloud consisted of spores of *Bacillus subtilis*. A Y-tube was attached to the end of the spray tube, one limb connected with an impinger, the other with a microfilter of resin wool. Flow through the latter was controlled at the distal end by a piece of capillary tube giving the same flow rate as that through the impinger. Both the impinger and the microfilter were backed by a microfilter to test for escaping spores. It will be observed from the table that the filtration efficiency with either device is of an exceptionally high order.

Table 2. *Comparative filtration efficiency of resin wool and impinger*

Comparison	Difference between total counts		S.E. of difference % for	Filtration efficiency (%)	
	R.W. – I.	%	for $P = 0{\cdot}05$	R.W.	I.
1	157	18·0 ± 6·7	9·5	99·99	99·80
2	257	31·4 ± 6·7	9·5	99·96	99·60
3	284	30·5 ± 6·4	9·2	99·99	99·54
4	335	39·6 ± 6·8	9·8	99·99	99·90
5	342	28·0 ± 5·7	8·1	99·98	99·36
6	363	40·0 ± 6·6	9·2	99·98	99·82
7	370	31·8 ± 5·8	8·3	99·99	99·15
8	397	40·0 ± 6·3	9·0	99·99	99·36

R.W. = Resin wool. I. = Impinger.

(3) *The assay of the collected cloud, and the reproducibility of results*

Dilution of the fluid in which the organisms have been collected or, in the case of the microfilter, in which the pad carrying the organisms has been shaken, are made in a suitable diluent which varies with the organism under test. For bacteria the method of Miles & Misra (1938) is used in estimating numbers. Great care is necessary in selecting the right medium and conditions for growth. When proper growth conditions are established for any organism the error of random sampling constitutes by far the largest source of discrepancy in the estimation. Normally, at least a thousand colonies are counted distributed on plates within the limits

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of 50–200 per plate. By this means differences greater than 10% between any two counts are significant.

With these procedures results are readily reproducible. Table 3 records the coefficient of variation obtained in each of nine experiments taken at random, where the cloud samples were collected by the liquid impinger. A spore suspension of *B. subtilis* in water was used throughout. The mean coefficient of 5·04% must be accepted as highly satisfactory. Similarly, Table 3 records results obtained with the delicate organism *Chromobacterium prodigiosum*. Relatively fresh suspensions of the organism were used. They were collected from the surface of agar and stored at ± 1° C. in high concentration (*c.* 1×10^{11}/ml.) in water, and diluted as required immediately before use in a phosphate buffer at pH 7·6. The coefficient of variation in this series is 5·98%. The mean coefficient of variation between the series of experiments with *B. subtilis* spores and *Chr. prodigiosum* is 5·73% (± 3·01%).

Table 3. *Coefficient of variation in sampling bacterial 'clouds'*

Exp. no.	*B. subtilis* spores (%)	*Chr. prodigiosum* (%)
1	5·40	4·04
2	6·40	1·82
3	2·70	5·87
4	10·40	3·23
5	6·10	2·31
6	3·43	3·48
7	4·17	7·49
8	4·37	5·17
9	2·41	5·31
10	—	7·91
11	—	11·95
12	—	6·60
13	—	7·40
14	—	14·90
15	—	2·16
Mean	5·04	5·98

(4) *Variations in the* A.S.F.

Whereas remarkable consistency of results may be expected if precisely similar conditions of test are used, wide divergencies in the A.S.F. can occur with relatively minor changes in the conditions of spraying, or of collecting a cloud. There are two factors of major importance in this respect:

(*a*) Physical characteristics of the material sprayed.

(*b*) The conditions under which the cloud is sampled.

Table 4 summarizes the results of an experiment in which Sky Blue in solution in water was sprayed alone, and in the presence of a suspension of anthrax spores used in varying concentrations. It will be seen that there is a significant fall in A.S.F. when spores are added to the dye in solution. This is most probably due to the physical change in the material to be sprayed. It will be noted, however, that over a wide range of spore concentration (10^5–10^8) no increasing fall in A.S.F.

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occurs. With the step in concentration to 10^9 spores, however, there is a further dramatic drop in A.S.F. and also in V.S.F. Frothing in the spray bottle tends to become a serious problem around these concentrations, and is probably responsible for trapping particles which otherwise would emerge and be collected. The significance of the difference between the V.S.F. and the A.S.F. is discussed in the following section. However, the difference is partly due to the fact that, as noted earlier, over 60 % of the particles emerging from the spray bottle and carrying dye in solution are $0{\cdot}2\,\mu$ in diameter, whereas the emerging spore has a diameter of approximately $1\,\mu$. Greater deposition of these larger particles is inevitable in transit from the point of emergence to the collecting point. An experiment demonstrating this phenomenon is summarized in Table 5.

Table 4. *The influence of physical characteristics of material sprayed on the spray factor*

Material sprayed	Conc. of spores/ml.	V.S.F. ($\times 10^6$)	A.S.F. ($\times 10^6$)	S.F.R. (%)
0·5 % S.B.	—	—	11·7	—
0·5 % S.B. + anthrax spore suspension	$1{\cdot}5 \times 10^5$	5·1	10·2	50
	$1{\cdot}8 \times 10^7$	5·3	10·8	49
	$1{\cdot}7 \times 10^8$	5·2	10·3	50
	$1{\cdot}1 \times 10^9$	2·3	6·2	38

Table 5. *The effect of sampling at different points along the spray tube*

Exp. no.	Material sprayed	Cloud samples at	Spray factor ($\times 10^6$) Viable	Absolute	S.F.R. (%)	Percentage ratio: end of tube / spray orifice
1	*B. subtilis* spores,	Spray orifice	8·5	12·9	66	77
	$1{\cdot}8 \times 10^8$/ml. + 0·5 % S.B.	End of tube	4·1	8·1	51	
2	*B. subtilis* spores,	Spray orifice	7·6	12·8	60	73
	$1{\cdot}1 \times 10^8$/ml. + 0·5 % S.B.	End of tube	3·5	8·0	44	

(5) *Variations in the* V.S.F.

It has already been noted that the filtration efficiencies of the impinger and the microfilter are at least 99 % for particles down to at least about $1\,\mu$. Variations occurring in V.S.F., therefore, are due either to low recovery efficiency, or to death of the organism in its path from the moment of shatter of the liquid, through the process of drying, to its collection either by impingement at velocities approaching that of sound, or by impaction on fibres and exposure to the desiccating influence of air passing at relatively high velocity. There are, therefore, both physical and biological factors involved.

(*a*) *Physical factors*

With certain organisms, recovery efficiency can be lowered as a result of physical as distinct from biological causes. Two possible sources of error in this respect have been noted. The first is probably unimportant, and well within limits of error of the biological assay methods. Thus, collection by either the liquid impinger

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or the microfilter probably leads to a small proportion of organisms being retained on the collecting surfaces. This one can prove by microscopic examination, for example, of the point of impingement in a liquid impinger. This error is probably less than 10 %. The second source of error can, with certain organisms, account for a 50–80 % discrepancy, and it has been designated the 'agitation phenomenon'. It appears that, by bubbling air rapidly through a liquid containing low concentrations (e.g. 10^4–10^6) of certain organisms, aggregation of the particles occurs. The subsequent assay, therefore, estimates clumps rather than single cells. Fortunately, many substances added to the suspension will prevent this phenomenon. The simplest and most effective that has been found is sodium alginate (Manucol) used in concentrations of about 0·1 %. Table 6 exemplifies the phenomenon and its prevention in the assay of clouds of *B. anthracis* spores with the liquid impinger.

Table 6. *Influence of Manucol on the agitation phenomenon*

Anthrax spore suspension	Spore conc. × 10^{-8} sprayed	Collecting fluid Na_2CO_3 buffer plus	Count/litre cloud collected (× 10^{-6})	Spray factor (× 10^6)
1	3·96	0·1 % Manucol	0·82	5·23
		0·04 % Manucol	0·79	5·04
		Buffer alone	0·56	3·56
2	4·74	0·1 % Manucol	0·87	4·62
		0·04 % Manucol	0·81	4·28
		Buffer alone	0·39	2·02
3	4·86	0·1 % Manucol	0·94	4·89
		0·04 % Manucol	0·89	4·57
		Buffer alone	0·48	2·48

(b) *Biological factors*

A discussion of biological factors is beyond the scope of this paper. In fact, one of the main purposes of the apparatus is to serve in the study of the behaviour of organisms as aerosols after growth under different conditions and suspension

Table 7. *Influence of suspending medium on the viability of a bacterial cloud of* Chromobacterium prodigiosum

Exp. no.	Suspension in	Concentration sprayed (ml. × 10^{-8})	Organism/litre cloud recovered from: impingers × 10^{-5}	Organism/litre cloud recovered from: microfilters × 10^{-5}	Percentage ratio: microfilter recovery / impinger recovery
1	P.B.	2·57	2·7	0·69	25
	P.B. + 2 % glycerin	2·54	2·5	3·14	126
2	P.B.	2·77	3·03	0·64	21
	P.B. + 2 % glycerin	2·70	2·93	3·3	113
3	P.B.	2·76	3·05	0·58	19
	P.B. + 2 % glycerin	3·43	2·72	3·13	115
4	P.B.	2·31	2·77	0·72	26
	P.B. + 2 % glycerin	2·86	2·07	2·9	140

P.B. = phosphate buffer at pH 7·6. Recovery by impinger of cloud from phosphate buffer = 100 %.

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in air at different temperatures and humidities. However, it may be noted that organisms vary very greatly in their power to survive as aerosols. Also, the suspending medium from which the aerosol is produced has a very important influence on the viability of the resulting cloud as is shown in Table 7. A thick suspension of *Chr. prodigiosum* in distilled water was diluted (*a*) in phosphate buffer, and (*b*) in phosphate buffer and 2 % glycerine to the same concentration. Clouds of each were produced and collected, by the Y-tube technique described above, by (*a*) impingers, and (*b*) microfilters. The protective influence of the glycerine is clearly demonstrated by the results obtained with the microfilters.

(6) *Response of animals to the aerosols produced*

The relative constancy with which clouds of the desired concentration could be produced made it reasonable to suppose that satisfactory dose response relationship in animals would be obtained. This has proved to be correct. One illustration, which has been more fully reported elsewhere (Elberg & Henderson, 1948), demonstrates the general trend. Groups of guinea-pigs were exposed to *Brucella suis* in varying concentration. The animals were sacrificed 1 month after exposure, and full cultural examination made for evidence of infection. Table 8 summarizes the results of four consecutive experiments. The regularity in response is dramatic. Statistical analysis of the regression line data gave an average weighted homogeneous slope value of 2·07 with a variance of 0·068. The I.D. 50 value expressed in terms of organisms per litre of aerosol was 240 with 5 % fiducial limits of 192–300.

Table 8. *The response-to-dosage data for guinea-pigs exposed to* Brucella suis *infection via the respiratory route*

Exp.	Estimated infecting dose*	No. animals infected	No. animals not infected	Animals infected (%)
1	26	8	10	44
	58	13	6	68
	97	15	5	75
	320	17	1	94
2	21	6	13	32
	49	11	9	55
	108	17	1	94
	270	18	0	100
3	22	6	13	32
	53	13	7	65
	108	15	2	88
	228	17	0	100
4	24	7	13	35
	40	13	7	65
	117	17	2	89
	270	19	0	100

* Twenty animals were exposed to each dose. The difference between this number and the total recorded in the table represents deaths during the holding period, due to failure of environmental control of 'guinea-pig pneumonia', prevalent at times in the stock.

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SUMMARY

A relatively simple and safe apparatus for the study of airborne infection is described.

Cloud sampling and assessment techniques are described and critically discussed.

Evidence is given concerning the satisfactory reproducibility of *in vitro* and *in vivo* assays.

Limitations to the usefulness of the apparatus are noted.

Dr D. D. Woods, now of the University of Oxford, was my collaborator in the early stages and played a major role in the development of technique.

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