A mobile form of the Henderson apparatus

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INTRODUCTION

The apparatus described by Henderson (1952) has been used extensively to produce aerosols consisting of single bacterial cells for the purpose of studying respiratory infection. In the original apparatus fine droplets are generated by a Collison spray and mixed with the main air flow in a 'spray tube' in which air from a silica gel bed is passing at 20 litre/min. This produces a relative humidity around 50 %. If the spray is fed from a bacterial suspension whose count is less than 10^9 /ml. it is unlikely that any droplet will contain more than one organism. In addition each droplet residue will contain solid matter derived from the suspending fluid.

Over the past two decades various alterations have been made by the author to improve the instrumentation, stability and general ease of operation. The use of the apparatus as an aerosol generator for such devices as the Rotating Drum (Goldberg, Watkins, Boerke & Chatigney, 1958) led to a requirement for humidity control in the air issuing from the mixing tube. More recently the introduction of the microthread technique (May & Druett, 1968) has called for a mobile piece of equipment so that cloud production and microthread preparation can take place near to other special equipment such as chambers containing germicidal material (Druett & Packman, 1968). A block diagram of the apparatus developed is shown in Fig. 1 and a general view in Plate 1.

DESIGN CONSIDERATIONS

The apparatus was designed to operate at 20–95 % R.H. at ambient temperature. The possible ambient temperatures considered were from 0 to 37°C. but it was assumed that temperature control, when required, would be provided by operating the mobile equipment in a temperature-controlled room.

The requirement of 20 % R.H. at 0°C. made inevitable an increase in the flow rate through the mixing tube. Since the water content of the air at 0°C and 20 % R.H. is 0.97 g./m³ and a single jet Collison spray gives a nominal output of 0.77 g./min. of water, a flow rate of 66 l./min. is minimal. The higher flow rate is advantageous even at room temperature (20°C), as under these conditions it allows the use of a 3-jet Collison spray throughout the working range which increases the aerosol concentration and renders the apparatus less vulnerable to jet blockage.

Fig. 1. Block diagram of the mobile Henderson apparatus.

Relative humidity control

The most convenient way to control relative humidity in a small dynamic cloud system is to mix, in known proportions, air containing water vapour at saturation ('wet air') with air which has passed through a silica gel bed ('dry air'). This is

achieved by passing the incoming air through a proportioning valve whose principle is shown in Fig 2. The air from the pumping system is led to the inlet channel in the sliding portion of the valve and passes thence through one of two slits into the appropriate outlet channel. The length ($a + b$) of the inlet channel is equal to that of one slit (a) plus the length (b) of the solid land separating the two slits. With the slide in the extreme left hand position all the air is diverted into channel 1

Fig. 2. Principle of the proportioning valve. In practice the slits of the proportioning valve are arranged to lie round a circle so that 'a' can be made longer without undue increase in bulk.

Fig. 3. Proportioning valve (drawn schematically).

and in the extreme right hand position it all passes to channel 2. At intermediate positions the air passes into both outlet channels proportionately to the length of the respective slits uncovered. In practice the slits are made as circular annuli and the general assembly is shown schematically in Fig 3. Provided that the pressure drop across the slits is large compared with that in the humidifier or drier, the total flow of air remains sensibly constant and the valve has a nearly linear characteristic. Air from the outlet channels passes to the appropriate flowmeters and thence to the humidifier and drier.

The drier

This consists of a 12 in. diameter $\times$ 18 in. high column of silica gel (or alumina) fitted with air spreading gauzes at the bottom.

The humidifier

In earlier models of this apparatus 'wet air' was produced by passing the airstream through a series of muslin screens dipped in water. This system has much to commend it: it is simple and has no moving parts and does not produce droplet residues in the airstream. The chief disadvantage was that the capillarity of the muslin deteriorated with age owing to the action of micro-organisms. This necessitated a periodic replacement of the muslin screens, which was time consuming.

Fig. 4. Diagram of humidifier.

The more elaborate and expensive spray system was adopted, the general layout of which is shown in Fig. 4. Air enters at the right of the humidifier by means of a side tube on the spray inlet tube, offset to encourage turbulence, and passes into the main chamber through the water spray produced by a 726A Allman No. 1 nozzle. The unevaporated water falls to the bottom of the chamber and is recirculated to the spray by a Stuart Turner pump. Since the temperature of evaporating droplets falls rapidly to the wet bulb temperature of the surrounding air, it is difficult to achieve high relative humidity without prolonged contact of the air with sprayed droplets if the water is initially at the ambient temperature. By raising the temperature of the water in the chamber, and consequently of the air in contact with it, the same relative humidity can be achieved at a higher temperature. The subsequent cooling of the air mass in the circuit of the apparatus raises its relative humidity. We found that humidifier temperatures 10°C. above ambient were

adequate to produce 99 % R.H. in the air circulating in the apparatus and for most purposes lower temperature settings were adequate. It is essential that the humidifier temperatures should not be raised more than is necessary as this leads to the condensation of water in the subsequent circuit of the apparatus and a consequent loss of control. The required heating is supplied by a 'Swan' 1 kW. kettle element controlled by a Sunvic Thermostat. A baffle plate is inserted between the water spray and the outlet tube of the humidifier to reduce the carry-over of water droplets into the emergent airstream, and the air-flow subsequently passes through a droplet trap. Non-return valves made of plastic (La Bas Tube Co. Ltd.) are inserted in the humidifier and drier circuits to prevent diffusion of moisture from the former to the latter when the machine is not in use.

Supply of conditioned air to spray tube

Air in the appropriate proportions from the humidifier and drier is mixed, and passes via the spray tube pressure control and cut-off No 2 (Fig. 1) to flowmeter 3, and thence via filter 4 to the spray tube. The spray pressure control is a manually controlled gate valve which allows excess air to escape from the circuit. Cut-offs 1 and 2 are connected hydraulically to the vacuum side of the pumping system. They prevent air from entering the spray tube and driving the pressure positive should any fault develop in the low pressure section of the circuit (*v. infra*). They also ensure that the pressure in the spray tube falls when the pumps are started or stopped, a property which is important if pathogenic organisms are used. The general mechanical design of these hydraulic cut-offs is illustrated in Fig. 5.

Flowmeter 3 indicates the volume of air entering the spray tube. If the spray tube pressure control is fully closed the reading of flowmeter 3 should equal the sum of that shown by flowmeters 1 and 2. It thus serves the dual function of indicating leakage in the humidifier and drier circuit under test, and the correct functioning of the control orifices under running conditions. Filter 4 protects the humidification section from accidental contamination but being upstream of the spray tube it should not become contaminated under normal conditions.

Spray circuit

Compressed air for the sprays is supplied by one side of pump 3, which is a dual compressor unit. The other side of the pump unit is used to augment the capacity of pumps 1 and 2 (*v. infra*.) The compressed air passes through a reservoir which smooths out the pulsations in the circuit, and a reduction valve, and is delivered at 26 lb./in.² to the spray circuit. As an alternative to the compressed air supply from pump 3, provision is made for the replacement of the air by other gases such as nitrogen from cylinders via a reduction valve as shown in Fig. 1.

Cut-off 3, which is hydraulically operated, interrupts the air-flow in the event of a failure in the low pressure section. Flowmeter 4 is specially calibrated to operate at 26 lb./in.² and indicates the free air output of the sprays at their operating pressure. From the flowmeter the air passes through one of two identical alternative circuits consisting of a solenoid valve, an aerosol filter, and a spray head. One spray reservoir contains distilled water ('water spray') while the other is charged with a

suspension of micro-organisms ('suspension spray'). A 3-position switch selects one or other, or neither of these circuits. The power supply is derived from the output (*a*, *b*, Fig. 1) of the pump control switch and renders both sprays inoperative immediately in the event of an electrical supply failure. The water spray is used in 'conditioning' the apparatus before an experiment. It is also used when replacement air at the correct humidity is required by ancillary apparatus to compensate for the air removed by sampling. As the suspension and water head sprays are identical, and the solid content of the suspension does not materially affect the spray output, the change over from one to the other does not alter the relative humidity of the air in the spray tube.

Fig. 5. Diagram of hydraulic cut-off.

The spray assembly

A general view of the spray assembly is shown in Fig. 6. All metal parts in contact with the suspension and the metal head (4) are made of stainless steel and the rest is of nickel-plated brass. Air at 26 lb./in.² enters through the central tube (6) and passes into the spray head (7), the machining details of which are shown enlarged at the right of the figure. The rubber seal (9) prevents air leakage at this point. The microbial suspension (11) is entrained by the jet and issues as an aerosol (12). The droplet size of the aerosol generated is dependent not only on the spray

head dimensions, but also on the distance (1 in.) between the point at which the spray jet emerges and the glass wall (1) of the envelope containing the suspension, which acts as a baffle. These essential dimensions are the same as in the original

Fig. 6. Details of spray assembly.

Henderson apparatus. The glass envelope can be made in various forms according to experimental needs. The form shown is useful when only short bursts of spraying are needed, and the quantity of suspension available is restricted. For long-period spraying a test tube shape is more suitable, as it allows a greater volume of suspension to be used, and this reduces the slow upward drift in cell concentration in the suspension due to water loss by evaporation from droplets which are impacted on the glass wall and do not escape as aerosol. The sliding extension piece (8) to the spray head which terminates in a short length of plastic tube allows adjustment to various envelope designs. The lower free end of the plastic tube should be cut with a zig-zag edge, as a clean cut is liable to form a seal against the surface of the glass. The top surface of the glass envelope is ground flat and sealed against the

metal head (4) by the compressive action of two free threaded metal rings (2, 3) acting through rubber washers (5). The downward thrust of the upper metal ring (3) operates on a smaller radius than the upward thrust of the lower metal ring (2) and this permits a small amount of deviation from parallelism between the planes of the glass and metal without leakage. The outer metal casing (10) is useful in transport and can be left *in situ* during operation. The author prefers to remove it so that the operation of the spray jets can be watched during an experiment. The small machine screw marked 'A' in the enlarged view of the spray head facilitates cleaning. The holes marked 'B' are threaded so that they can be closed by a machine screw, reducing the number of jets in operation from 3 to 2 or 1 if required.

The spray tube

This consists of approximately 30 in. of 2 in. diam. tubing. The pipes through which the water and suspension spray enter terminate near the axis of the tube immediately in front of the tube carrying the main flow from filter 4. The spray tube can be separated into two halves for convenience in autoclaving. The distal end from the spray is fitted with a side tube through which aerosol can be extracted. If desired, ports for animal exposure can be added as described in Henderson's paper. For some bacterial suspensions sprayed in air at high relative humidity the spray tube length is inadequate for equilibrium to be attained between the droplet and the surrounding air. This is unimportant in microthread work or when holding apparatus, such as the Goldberg rotating drum, is to be used, since adequate time for equilibrium will be available subsequently. In inhalation studies the droplet size is of primary importance and special tube designs would be needed to ensure that equilibrium had been attained before inhalation.

To fill large holding devices which are being used repeatedly it is convenient to divert the main air flow of the apparatus at the point marked Z (Fig. 1) through a modification to the pipe work (see insert) and leave the holding device permanently attached. The portions of the tubing marked 1, 2, 3 are made of rubber. In normal operation 2 and 3 are closed by clips but 1 is left open. To divert the flow, clip 2 is released. Simultaneously 1 is closed and 3 opened. The flow now passes through the holding apparatus. After the appropriate time interval 1 is opened and 3 closed simultaneously, and subsequently 2 closed, trapping the aerosol in the holding device. The same process is used to remove the residual aerosol from the holding device at the end of the experiment, the Henderson apparatus being run with the suspension spray turned off for this purpose.

The hygrometer

On leaving the spray tube the air stream passes over a wet and dry bulb hygrometer and through filter 1. The hygrometer is placed upstream of the filter, as experience has shown that erroneous readings are obtained at high humidities if the filter precedes the hygrometer. Various forms of hygrometer have been tried but the wet and dry bulb system has been chosen since it is easy to sterilize by immersion of the contaminated parts in hypochlorite solution, and provided the 'wick' is replaced after each run it does not become 'poisoned' by deposition of the

passing aerosol. To obtain correct humidity readings the air flow over a standard hygrometer thermometer should not be less than 10 ft./sec.

Control orifices and filters

The flow in the spray tube is controlled by orifices 1 and 2 to a flow rate of 66 l./min. The flow rate of orifice 2 is chosen to match the flow rate which it is intended to use in the sampling circuit so that by opening one circuit and closing the other the flow through the spray tube is maintained at a constant value. The standard Porton impinger (Henderson, 1952) uses a flow rate of approximately 11 l./min. and acts as its own critical orifice. It is therefore convenient to set control orifice 2 to this value, and to install a sample orifice of the same value to control the flow through other sampling devices such as the 'Sow' (May & Druett, 1968). Orifices 1, 2 and 3 are of the Hartshorn type, the design of which has been described (Druett, 1955), and will give constant flow for pressure drops greater than 6 in. Hg. If a critical flow impinger is used in the sampling circuit, the pressure drop across the orifice must exceed 15 in. Hg. and owing to the limited capacity of the pumps this places a severe limitation on the amount of air or other gas which can be admitted at the controlled gas leak. For some purpose it may prove more convenient to use a separate external pumping system.

Filter 7 prevents contamination of orifice 3 and the pumping circuit. Filter 2 removes any penetration from filters 1 and 7 (which have a penetration of less than 0.002 % as determined by the sodium flame test) and provides second line protection to the pumps in the unlikely event of filters 1 or 7 failing.

The pump vacuum gauge and controlled gas leak

The pump vacuum gauge is calibrated in lb./in.² and inches of mercury and indicates whether sufficient vacuum exists for the correct functioning of the control orifices (v. supra).

The controlled gas leak is provided at the pump intake to permit the steady changing of the air (or other gas) in the circuit. This is essential for oxygen replacement if animals are to be exposed: a small change is desirable in other cases so that any vapour released by a component cannot accumulate. Lengths of capillary tubing of appropriate bore form suitable orifices for this purpose.

OPERATION

Preliminary testing

Both sprays are charged with 20 ml. of sterile distilled water and connected into the circuit. The apparatus is brought into operation by pressing the pump control switch. The two spray heads are now tested. This is done by setting the spray control switch successively to positions 1 and 3 with the spray gauge reading adjusted to 26 lb./in.² and taking readings from flowmeter 4. This should read 2.4 l./min. multiplied by the number of jets in use: a lower reading indicates jet blockage and a higher one leakage at one of the connexions or washers. At the same time a visual inspection of the spray emerging from the jet should be made as malfunction

due, for instance, to a blocked liquid feed tube can readily be detected. Blocked air jets can be remedied with a No. 80 drill: wire should not be used, as it scratches and distorts the bore of the air jet and prolonged use leads to erroneous flow readings. After both sprays have been checked the apparatus is switched off, the water removed from the suspension spray and the glass envelope (1) (Fig. 6) dried. It is not necessary to ensure complete sterility of the spray head, since the bacterial suspension added to it has a vastly higher bacterial count than any chance contaminant and the suspension is discarded after use so that no opportunity is afforded for the contaminant to grow in the suspending fluid.

The thermostat of the humidifier is adjusted to about 5°C. above the ambient temperature, and its heater and spraying unit switched on. About 15 min. should be allowed for thermal equilibrium to be established. After this period has elapsed the apparatus is started with the entry to the spray tube from the suspension spray closed and the water spray in operation. The spray tube pressure is adjusted to -1 in. water gauge by means of the spray tube pressure control. The humidity is adjusted by means of the proportioning valve until the appropriate value is obtained. An approximate setting can be obtained by calculation. If a relative humidity of 60% at 20°C. is required in the spray tube and flow meters 1 and 2 indicate together a total flow of 70 l./min., and an allowance of 6% R.H. is made for the water discharged from the water spray at this temperature, then flow meter 1 should be set to $[(60 - 6) \times 70] / 100 = 37$ l./min. Further trimming adjustments are made as necessary.

Safety

Up to this point the apparatus has not been contaminated with micro-organisms and no safety hazard is involved. The appropriate safety precautions are now taken. While in normal use no aerosol escapes from the apparatus and every effort has been made to guard against the effects of electrical and mechanical failure, the 'second line of defence' recommended by Henderson of wearing respirator, hood, gown and gloves is adopted whenever pathogens are sprayed. Similar protection is afforded to the general population by ensuring that the room used is provided with an effective air extract system fitted with an efficient particulate filter. The use of chemical disinfectant aerosols advocated by Henderson is not favoured by the author since these act too slowly to provide any useful protection to the operator and, being for the most part persistent on surfaces, their vapour tends to find its way into apparatus and vitiate subsequent holding experiments. Strategically placed germicidal lamps, if well maintained, offer a more rapid safeguard should the need arise, and these can be run with the operator in the room, since he is fully covered.

The suspension spray

The appropriate bacterial suspension is placed in the reservoir of the suspension spray. The block on the entry to the spray tube is removed and the spray assembly connected to the apparatus. A check is made that the spray tube vacuum gauge is

reading - 1 in. water and that the hygrometer reading is correct. With full safety precautions in force the 3-way spray control switch is brought to position 1 and the bacterial aerosol is available for use.

Sterilization

The apparatus is arranged so that the contaminated portion (i.e. between filters 4, 5, 6 and filter 1) can be removed for autoclaving. The wet and dry bulb thermometers (which would be damaged by autoclaving) are placed in 5% hypochlorite solution for 15 min. Alternatively a gaseous disinfectant can be used provided it is non-persistent. Pleated fibreglass paper filters will withstand autoclaving without breakdown but should be subject to periodic testing. Alternatively a jig type filter may be used which can be recharged after autoclaving.

CONCLUSION

This apparatus has been extensively used and found to be capable of running unattended for considerable periods. It can be moved from one site to another and be brought into operation within minutes. It has been transported on lorries and towed slowly for short distances behind vehicles on its own wheels and continued to function. Several variants of this apparatus have been built and formed the basic utility for the work described, *inter alia*, by Harper (1961), Harper & Hood (1962), Hood (1963), May & Druett (1968) and Druett & Packman (1968).

SUMMARY

A modification of the Henderson apparatus is described which is mobile and which can produce bacterial or viral aerosol in air whose relative humidity can be chosen in the range 20-95%. The apparatus forms a basic utility for microthread, rotating drum or other aerosol studies.

Much help and advice has been given by Messrs. J. F. H. Peel and D. J. Garriock of the Engineering Section M.R.E. throughout the development of this apparatus. The proportioning valve is a modification of one originally used for a similar purpose by Mr K. R. May. Thanks are due to Messrs. G. J. Harper and A. M. Hood for criticism of the script.

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EXPLANATION OF PLATE 1

General view of the mobile Henderson apparatus.