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Aerosol sampling methods for the virus of foot-and-mouth disease and the measurement of virus penetration through aerosol filters

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INTRODUCTION

Efficient sampling methods are necessary for the study of airborne transmission of the virus of foot-and-mouth disease (FMD). Experiments are reported here which show that aerosols of the virus of FMD like those of a few other viruses (e.g. see Rosebury, 1947) can be reproducibly and quantitatively sampled by slight modification of standard aerosol sampling methods. The application of these methods to the determination of the penetration of virus through materials used in air filtration systems is also described.

MATERIALS AND METHODS

Virus suspensions. Two strains were used, Dutch 57 (type 0), grown in surviving fragments of cattle tongue epithelium and M 11 (type 0), grown in pig kidney monolayers (Sellers, 1955). Suspensions for spraying were 1:10 dilutions of the stock virus suspensions in Tyrode's solution containing 0·5% lactalbumin hydrolysate (TLH), equal parts of TLH and 0·04 M phosphate buffer, pH 7·6 (PB) or PB alone.

Virus assay. Samples were assayed by intraperitoneal inoculation of appropriate dilutions in PB or phosphate buffered saline (PBS) into unweaned mice (Skinner, 1951) or, in a few experiments with the M 11 strain, by plaque titration on pig kidney monolayers (Sellers, 1955).

Concentration of virus by adsorption. Concentration of the sample before inoculation facilitated the detection of small amounts of virus. This was achieved by adding an adsorbent, Attaclay, found to be an efficient adsorbent for the virus of FMD (Cartwright & Thorne, 1959), to the virus suspension, sedimenting the adsorbent by centrifugation (3000 r.p.m., 5 min.) and suspending the deposit in a small volume of diluent before inoculation. Because of the generally small concentration of extraneous substances in the sampler liquids small amounts of adsorbent suffice for complete adsorption. Table 1 shows the virus recovered after adsorption, sedimentation and resuspension by different concentrations of Attaclay from a virus suspension of low titre and negligible extraneous matter (a 1/50,000 dilution of M 11 stock virus, in PB or PBS).

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While the results indicate that as little as 0·05 mg./ml. is probably effective 1 mg./ml. has been used as a standard quantity to ensure an excess of adsorbent in case samples with a higher content of extraneous material were encountered. Concentration factors of 50–100 fold were obtainable. Attaclay alone inoculated intraperitoneally at a concentration of 50 mg./ml. was harmless for unweaned mice.

Filter materials. Representative samples of filter media used in the manufacture of several types of aerosol filter were kindly supplied by Vokes Ltd., Guildford. The materials tested with manufacturing tolerances for methylene-blue test penetration values determined by Vokes Ltd., at corresponding recommended air flow velocities, were: asbestos-cotton (0·003 %; 2·75 ft./min.), 55 glass fibre paper (0·05 %; 4·6 ft./min.), glass wool (0·35 %; 14·5 ft./min.) and 44 asbestos fibre paper (5·0 %; 4·6 ft./min.). Disks of the materials backed by a perforated plate were held in glass Seitz filter-holders appropriately gasketed and sealed to prevent air leaks.

Membrane filters and filter-paper samplers. 6-cm. 'Oxoid' cellulose acetate membrane filters and Whatman No. 1 filter-paper disks were backed by a supporting grid and mounted in Seitz filter or Gradocol membrane-holders.

Table. 1. *The adsorption of low-titre virus by Attaclay*

Conc. of Attaclay (mg./ml.)	Total virus recovered from deposit (p.f.u.)
1	$1{\cdot}3 \times 10^3$
0·5	$1{\cdot}2 \times 10^3$
0·1	$4{\cdot}8 \times 10^2$
0·05	$8{\cdot}0 \times 10^2$
Total virus content of original suspension	$1{\cdot}0 \times 10^3$

Impingers. The following liquid impinger samplers were used:

(*a*) Standard Porton impingers described by Henderson (1952) with critical flow velocities of 10–11 l./min.

(*b*) Impingers constructed at this Institute of the type illustrated (Fig. 1) eliminating the cone joint of the Porton impinger to provide a more robust, rapidly constructed and inexpensive sampler for use in cattle boxes where breakage rate might become high. These impingers had critical flow velocities of 5–10 l./min. (capillary diameter 0·7–1 mm., length 7 mm.).

(*c*) Impingers similar to (*b*) but provided with up to 8 jets directed at the walls of the container for sampling at rapid rates from cattle boxes (Fig. 1). The walls of the impingers (*b*) and (*c*) were dimpled inwards and the inlet tubes correspondingly baffled at several points to increase efficiency of capture of small particle aerosols and reduce splashing losses when sampling at high flow rates. The impinger liquid was generally PB but PBS was also used with similar results.

Flow meters. These were variable-area flow-meters manufactured by G. A. Platon Ltd., Croydon.

Experimental arrangement and procedure

A diagram of the apparatus used for testing samplers and filter penetration is shown in Fig. 2. To reduce the danger from escaping infective aerosols the necessary air flow was produced by suction with a rotary vacuum pump (Edwards RB4)

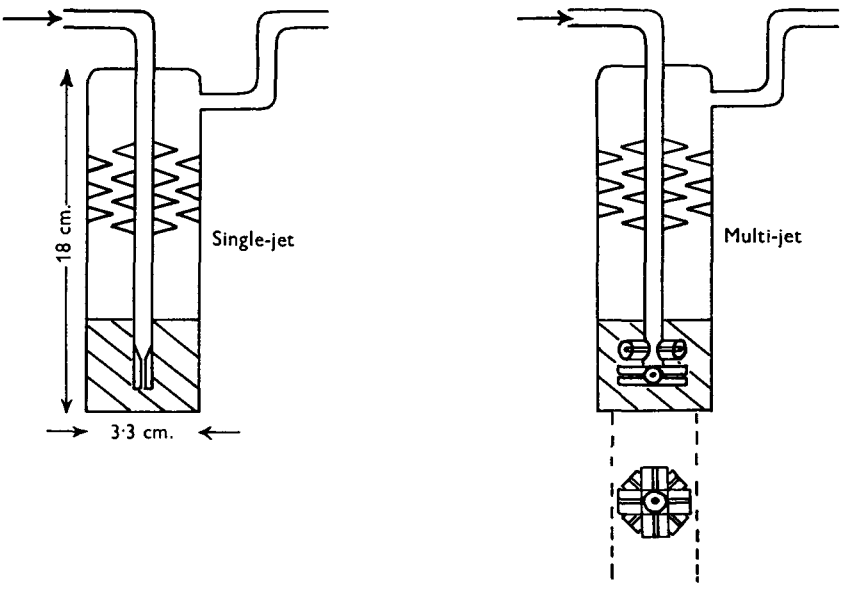

Fig. 1. Construction of one-piece impingers.

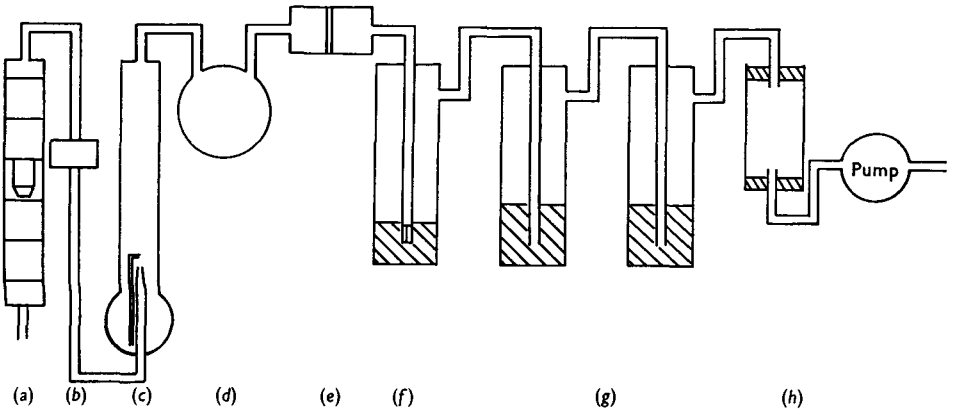

Fig. 2. Apparatus for producing and sampling aerosols.

through the following components in turn: (*a*) flow-meter; (*b*) cotton wool dust-filter of negligible air resistance; (*c*) the aerosol generator, an atomizer or nebulizer of the construction shown and capacity 15 ml. with the vertically directed air flow aspirating fluid from the reservoir through a capillary tube into a glass column about 12 in. long which continuously refluxed the fluid and served to remove the larger particles (for the production of very fine sprays, the column can be dimpled

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inwards to remove large particles as described for the impingers); (*d*) a 2-litre flask which further filtered out large particles and allowed drying of the aerosol droplets, as well as acting as a small storage vessel for the study of an aerosol cloud. The particle size distribution of the aerosol emerging at this point was not determined. However, microscopic examination of samples collected on glass slides indicated that the majority of the particles were much below 1 μ in size; (*e*) a filter-holder when filters were to be tested; (*f*) the sampling device; (*g*) a sulphuric acid-trap to inactivate any virus which might be carried through the sampler; and (*h*) a soda lime-trap to prevent acid fumes passing into and corroding the pump.

Air at atmospheric pressure was let into the apparatus before switching off the pump to prevent suck-back of impinger and trap liquids. To obtain air-flow rates less than the critical flow rate determined by the impinger capillary, the filter or sampler under test was by-passed with a rubber tube and while constant air flow through the atomizer was maintained the flow rates through the two paths were adjusted to give the required rate by screw clamps on the rubber connecting tubes. Rapid flow rates, e.g. for testing multiple impingers, were obtained by mixing the aerosol air stream with air entering the system through a separate orifice. A dummy test arm was sometimes included in parallel with the system under test to allow prior adjustment of flow rate.

The efficiency of impingers was tested as follows: a dye, Rhodamine (I.C.I. Ltd.), which was without effect on the infectivity of the virus, was incorporated at a concentration of 0·5 % in the virus suspension being sprayed. By adsorptiometric measurements at 560 mμ of the concentration of the dye in the impinger liquid, the volume of the original virus suspension held by the sampler was calculated. The pump was turned on and after adjustment of the flow velocity the aerosol was collected by the impinger under test for a period of 1–15 min. The impinger liquids were then removed and assayed for dye and virus content. The volume of the original virus suspension received by the impinger was calculated and compared with the recovery of virus to determine any losses of viability, or losses by other processes, e.g. adsorption within the impinger. The fraction of incident virus passing through the impinger (filtration efficiency) was estimated by the ratio of the titres of two identical impingers connected in series. The efficiencies of other impingers were then determined by comparison with an impinger of known efficiency under the same operating conditions.

Membrane-filter or filter-paper samplers were tested in a similar manner. After the sampling period the disks were removed and suspended in PBS, PB or a solution of the detergent sodium dodecyl sulphate (Cartwright & Thorne, 1959) in the same buffer, agitated and partially broken up by a glass rod for 5–10 min. before centrifugation and removal of the supernatant liquids for assay of dye and virus content.

The penetration of virus through filter materials was determined using impinger samplers. The ratio of the virus content of the impinger fluid after sampling for 5 min. in the presence and absence of the filter-pad at constant air-flow rate gave the penetration value. The tests were made at the recommended air-flow rate and at a flow rate determined by the critical flow velocity of the impinger (10 l./min.).

RESULTS

Impinger sampling efficiency

Table 2 gives the results of an experiment comparing the recovery of virus by a 10 l./min. Porton impinger from aerosols sprayed from different media with the volume of virus suspension trapped by the impinger, estimated by dye adsorption and the titration of the spray suspension.

The amounts of virus and dye recovered from a second impinger in series were less than 1 % of those from the first. The impinger was, therefore, more than 99 % efficient in filtering out virus and losses of virus within the impinger were absent within the accuracy of the titration method (S.E. ± 0·2) for the sampling period used.

Table 2. *The recovery of virus and dye from impinger liquids*

Medium	Titre of impinger liquid (ID_{50}/ml.)	Titre calculated from optical density
TLH/PB 1:1		
Control	3·8	—
+ Tracer	3·4	3·9
+ Tracer	3·5	3·9
PB		
Control	4·1	—
+ Tracer	3·9	4·0
+ Tracer	3·5	3·9

Table 3. *The dependence of recovery on flow rate*

Flow rate (l./min.)	Proportion of positive reactions at dilutions of impinger liquids			Titre of impinger liquid (ID_{50}/ml.)
	10^{-3}	10^{-4}	10^{-5}	
10	20/20	14/20	0/20	$10^{5·6}$
2	20/20	3/21	0/20	$10^{5·05}$

Influence of flow rate on Porton impinger efficiency

Experiments with Porton impingers at flow rates below 10 l./min. showed that recovery was complete at all flow rates used. In the experiment of Table 3 the difference in titre was consistent with the five-fold difference in flow rate.

Efficiency of one-piece impingers

The recovery of virus by one-piece single jet impingers of various critical flow velocities up to 10 l./min. was equivalent to that of the Porton impinger. Multiple jet impingers were tested up to flow rates of 50 l./min. with the M 11 strain and found to be fully efficient within limits of error (Table 4). The air-flow rate through the atomizer was constant in each experiment so that the input of virus was always the same.

Membrane-filter and filter-paper samplers

The recovery of virus from membrane-filter and filter-paper samplers is compared with that from impingers in Table 5. Membrane filters, using SDS to elute the adsorbed virus, were equivalent in efficiency to the impinger, but filter-paper, even using SDS, was less efficient. The necessity for using an agent to promote elution from both types of filter was shown by the poor recoveries when either PB or

Table 4. *The recovery of virus by single and multi-jet impingers*

	Air-flow rate through impinger (l./min.)	Titre of suspension sprayed (p.f.u./ml.)	Impinger liquid titre (p.f.u./ml.)	Sampling time (min.)
Porton impinger	5·0	1×10^6	$6{\cdot}5 \times 10^2$	5
One piece—1 jet	5·0	1×10^6	$6{\cdot}4 \times 10^2$	5
One piece—6 jet	17·5	1×10^6	$7{\cdot}7 \times 10^2$	5
One piece—8 jet	20·0	1×10^6	$1{\cdot}0 \times 10^3$	5
One piece—8 jet	50·0	1×10^6	$8{\cdot}0 \times 10^2$	5
Porton impinger	5·0	2×10^5	$2{\cdot}4 \times 10^2$	5
One piece—6 jet	15·0	2×10^5	$2{\cdot}4 \times 10^2$	5
Porton impinger	10·0	$1{\cdot}9 \times 10^7$	$1{\cdot}3 \times 10^4$	3
One piece—1 jet	10·0	$1{\cdot}9 \times 10^7$	$1{\cdot}2 \times 10^4$	3

The volumes of sprayed suspension trapped by the impinger estimated from the virus titres (about 1×10^{-2} ml.) were equivalent to those calculated from dye absorption measurements in all cases.

Table 5. *The recovery of virus by membrane-filter and filter-paper samplers*

Virus strain	Sampler	Flow rate (l./min.)	Sampler medium and volume	Total virus recovered (ID_{50} or p.f.u.)	Time (min.)
Dutch O	Impinger	8	10 ml. PB	$10^{3{\cdot}9}$	1·5
	Membrane-filter	8	1 ml. 1 % SDS	$10^{3{\cdot}6}$	1·5
	Filter-paper	8	1 ml. 1 % SDS	$10^{3{\cdot}4}$	1·5
M 11	Impinger	5	10 ml. PB	$10^{4{\cdot}6}$	3·0
	Membrane-filter	5	1 ml. 1 % SDS	$10^{4{\cdot}5}$	3·0
	Membrane-filter	5	1 ml. 0·1 % SDS	$10^{4{\cdot}1}$	3·0
	Membrane-filter	5	10 ml. PB	$10^{3{\cdot}0}$	3·0
	Filter-paper	5	1 ml. 1 % SDS	$10^{3{\cdot}5}$	3·0
	Filter-paper	5	10 ml. PB	$10^{2{\cdot}4}$	3·0
M 11	Impinger	5	10 ml. PBS	$10^{5{\cdot}4}$	3·0
	Membrane-filter	5	10 ml. PBS	$10^{2{\cdot}5}$	3·0
Dutch O	Impinger	Flask containing aerosol exhausted (max. rate 10 l./min.)	10 ml. P.B.	$10^{3{\cdot}8}$	5·0
	Membrane-filter	Flask containing aerosol exhausted (max. rate 10 l./min.)	1 ml. 1 % SDS	$10^{2{\cdot}3}$	5·0

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particularly PBS was used alone as the eluting medium even though all adsorbed dye was eluted by these reagents. The recovery was about twice as great with 1 % as with 0·1 % SDS. It is possible by eluting the virus in a small volume of detergent (see Table 5) to increase the sensitivity of the method and this is of value when dealing with aerosols of low titre.

In the final experiment in Table 5 an aerosol was introduced into a flask from which it was pumped through an impinger or membrane-filter until the flask was exhausted. The reasons for the much lower recoveries in this experiment are not clear, but inactivation by drying *in vacuo* may be a factor involved.

Table 6. *Titrations of virus penetrating filter materials*

Filter material	Flow rate (ft./min.)	Proportion of positive reactions at dilutions of impinger liquid					
		10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
		Excess flow rate					
Control	23	—	—	20/20	20/20	14/20	0/20
Glass wool	23	40/40	20/20	9/10	0/10	—	—
Asbestos-cotton	23	41/41	20/20	6/10	0/10	—	—
44 asbestos paper	23	40/40	20/20	10/10	—	—	—
55 glass fibre paper	23	7/41	0/20	0/10	—	—	—
		Recommended flow rates					
Control	4·6	—	20/20	18/21	20/20	3/21	—
Glass wool	14·5	13/19	3/21	1/20	—	—	—
Asbestos-cotton	2·75	10/20	1/20	0/10	—	—	—
44 asbestos paper	4·6	2/10	0/20	0/20	—	—	—
55 glass fibre paper	4·6	0/41	0/10	—	—	—	—

Table 7. *Methylene blue and virus penetration values for filter materials*

Filter material	MB penetration tolerance at recommended flow rate (%)	Virus penetration at recommended flow rate (%)	Virus penetration at excess flow rate (%)
Glass wool	0·35	0·025	2·5
Asbestos-cotton	0·003	0·05	0·8
44 asbestos paper	5·0	0·01	$\geqslant 4{\cdot}0$
55 glass fibre paper	0·05	$<0{\cdot}0012$	0·002

Virus penetration through filter materials

Virus titration results for the penetration of strain Dutch 57 through a single uncompressed thickness of material at excess and recommended flow rates are given in full in Table 6 to indicate the reliability of the penetration results obtained.

The calculated penetration figures with the manufacturing tolerances for methylene blue penetration given by Vokes Ltd. for the recommended flow rates are given in Table 7.

Glass fibre paper was the most effective filter material for the virus of FMD. Besides having the lowest penetration value the increase in penetration for a

fivefold increase in flow rate was very small. The very low penetration was shown by titration of a mixture of virus suspension and ground filter material not to be due to chemical inactivation of the virus by the filter material.

DISCUSSION

By combining the impinger method with an appropriate assay the sampling of laboratory-generated aerosols of the virus of FMD has been shown to be a feasible procedure which allows quantitative estimation of airborne virus. This method of sampling would probably be suitable for use in surveying laboratory operations which might set up undesirable infectious aerosols and for the study of the survival and physical behaviour of virus aerosols.

In addition to their use in preventing virus escape, via ventilation installations, the filter materials tested can be used to prevent contamination of pumps used in the sterilization or freeze-drying of infective material or on outlets emitting infective aerosols. The choice of material will depend on the nature of the filter, the flow rate desired and the tolerated virus penetration. Glass fibre paper is a very convenient material from which to manufacture filters in the laboratory. The materials were tested here as flat uncompressed single thicknesses; somewhat better performances would be expected from the glass wool and asbestos-cotton materials when folded and compressed in practical filters for dealing with large volumes of air. Since the size of dry aerosol particles, except those from virus suspensions in pure water, is probably largely a function of the composition of the suspension medium and not of the virus particle size it is possible that similar values of penetration through these materials would be found for other viruses. Glass fibre papers and glass wool tested with T3 bacteriophage by Decker, Geile, Moorman & Glick (1951) and Sadoff & Almof (1956) gave values of penetration consistent with those found for the virus of FMD.

Experiments to establish the relationship of aerosols to the transmission of natural infection are facilitated by the methods described. Preliminary investigations in which air in the vicinity of cattle infected with the virus of FMD has been sampled by assemblies of impingers in parallel (Thorne & Hyslop unpublished) indicate that the methods described in this paper may yield results which could clarify this relationship.

SUMMARY

Single and multi-jet liquid impingers and membrane-filters were found to be efficient sampling devices for aerosols generated from suspensions of the virus of foot-and-mouth disease (FMD). Concentration of the aerosol samples with an adsorbent, Attaclay, facilitated the detection of small amounts of virus. Sodium dodecyl sulphate could be used for elution as the virus of FMD is resistant to this anionic detergent.

The penetration of these aerosols through various air filtration media was determined using impinger samplers. A glass fibre paper was found to be the most efficient with a virus penetration of less than 0·001 %.

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