APPLIED MICROBIOLOGY, Nov., 1965 Vol. 13, No. 6
 Printed in U.S.A.

Sampling Submicron T1 Bacteriophage Aerosols

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Received for publication 3 June 1965

ABSTRACT

HARSTAD, J. BRUCE (Fort Detrick, Frederick, Md.). Sampling submicron T1 bacteriophage aerosols. Appl. Microbiol. **13**:899–908. 1965.—Liquid impingers, filter papers, and fritted bubblers were partial viable collectors of radioactive submicron T1 bacteriophage aerosols at 30, 55, and 85% relative humidity. Sampler differences for viable collection were due to incomplete physical collection (slippage) and killing of phage by the samplers. Dynamic aerosols of a mass median diameter of 0.2 μ were produced with a Dautrebande generator from concentrated aqueous purified phage suspensions containing extracellular soluble radioactive phosphate as a physical tracer. There was considerable destruction of phage by the Dautrebande generator; phage titers of the Dautrebande suspension decreased exponentially, but there was a progressive (linear) increase in tracer titers. Liquid impingers recovered the most viable phage but allowed considerable (30 to 48%) slippage, which varies inversely with the aerosol relative humidity. Filter papers were virtually complete physical collectors of submicron particles but were the most destructive. Fritted bubbler slippage was more than 80%. With all samplers, phage kill was highest at 85% relative humidity and lowest at 55% relative humidity. An electrostatic precipitator was used to collect aerosol samples for particle sizing with an electron microscope. The particle size was slightly larger at 85% relative humidity than at 30 or 55% relative humidity.

The literature on microbial aerosols has been concerned mainly with bacterial aerosols (Wolf et al., 1959). Less is known about viral aerosols (Webb, Bather, and Hodges, 1963), particularly with regard to the production, particle sizing, and sampling of submicron aerosols of viruses. Submicron aerosols have been studied extensively, but knowledge is limited almost entirely to aerosols of inert materials. Extremely fine aerosols in the millimicron size range (<0.1 μ) have been produced from dilute solutions of dyes and salts and from colloidal suspensions (Dautrebande, 1962). Stern et al. (1959) generated homogeneous millimicron aerosols from dilute colloidal suspensions with a modified Vaponefrin nebulizer. Their technique was unique in that the colloids were highly purified viral suspensions. The concentrations of the suspensions were adjusted by dilution with water so that the chance of two virus particles being present simultaneously in a single evaporating water droplet was about 1 to 100. Morris et al. (1961) evaluated an electrostatic precipitator for sampling monodispersed T3 coliphage aerosols produced with a modified Collison atomizer from phage broth lysates diluted extensively with water so that the aerosols consisted principally (95%) of single phage particles.

Physical tracers such as radioactive isotopes, dyes, and bacterial spores have been used to study the behavior and sampling of microbial aerosols. Harper, Hood, and Morton (1958) and Miller et al. (1961) used radioactive isotopes and bacterial spores to study the survival of bacterial aerosols. Harper (1961) used radioactive phosphate to study the survival of airborne viruses. Tyler, Shipe, and Painter (1959) used physical tracers with bacterial aerosols to show that slippage of the aerosol particles through the samplers, particle size discrimination, and destruction of bacteria during the sampling process are sources of sampler differences.

This paper reports on the generation, sampling, and particle sizing of submicron aerosols of T1 phage. The aerosols were produced with a Dautrebande aerosol generator from concentrated aqueous suspensions of purified phage containing soluble radioactive phosphate as a physical tracer. Five air-sampling devices were compared for the physical and viable collection of submicron aerosols at relative humidities of 30, 55, and 85% and a temperature of 24 C. The samplers were all-glass impingers (AGI-4), capillary impingers, Chemical Corps Type 6 filter papers, MSA 1106BH filter papers, and fritted bubblers.

MATERIALS AND METHODS

Phage concentration and purification. T1 and T3 bacteriophage, two of the well known T series of phages of *Escherichia coli*, were studied. These phages are relatively stable to chemical and physical manipulations and are relatively easy to obtain in pure form. Aerosolization of concentrated aqueous purified suspensions of both of these phages resulted in aerosols of small particles well within the submicron size range ($< 0.3\ \mu$). However, the aerosols are subject to biological decay, and the purification required to obtain submicron aerosols is detrimental to the survival of the airborne phage. Studies with submicron T3 phage aerosols were discontinued when tests revealed that T1 phage was much more stable in air, especially at the lower relative humidities.

An electron micrograph of a T1 phage particle prepared by the air-drying technique is shown in Fig. 1. It is tadpole-shaped, with a slender tail about 150 mμ long attached to a hexagonal head about 60 mμ in diameter. Aqueous purified phage suspensions were used to produce submicron aerosols. The suspension had to be free of soluble and particulate contaminants to produce an aerosol of minimal particle size. Clean phage suspensions were prepared by concentrating and purifying large volumes of broth lysates by differential centrifugation and washing with distilled water.

T1 phage lysates were produced in double-strength nutrient broth (Difco). Batches of 5 liters in 18-liter carboys proved convenient. The seed inoculum of *E. coli* strain B (ATCC 11303) for a carboy was prepared in 100 ml of the same medium in a 500-ml Erlenmeyer flask and incubated at 37 C on a shaker for 16 hr. The carboy, previously warmed to 37 C, was then inoculated with 100 ml of the seed *E. coli* culture and incubated at 37 C with aeration on a shaking machine. After 3.5 hr of incubation, the *E. coli* culture had reached the end of the logarithmic phase of growth (10^9 cells per milliliter). Crude phage lysate, clarified by low-speed centrifugation, was added in the ratio of 2 to 5 phage particles per bacterium, and the carboy was shaken 4 to 5 hr. Aqueous purified phage suspensions were not used for seeding *E. coli* cultures because higher phage yields were obtained when broth lysates were used for inoculation (Puck, 1949).

The crude lysates titering 10^{11} phage particles per milliliter were clarified at low speed ($10{,}000 \times g$) for 10 min in a Sorvall SS-1 centrifuge (Iván Sorvall, Inc., Norwalk, Conn.) to remove bacterial and large-particle debris. The supernatant fluid was decanted and centrifuged at high speed ($60{,}000 \times g$) for 25 min in a no. 30 rotor in a model L Spinco preparative ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) to sediment the phage component. The supernatant fluid was drawn off with a flat-tipped needle until the level of the liquid remaining in the bottom of the centrifuge tube was just above the virus pellet. The pellets were resuspended in the bottom supernatant fluid by shaking and were treated with 5 μg/ml of each of the enzymes deoxyribonuclease and ribonuclease (Calbiochem) for 2 to 4 hr at 37 C in the presence of an $MgSO_4$ concentration of 0.003 M and at a final *p*H of 6.6. The enzymatic digestion of extraneous deoxyribo-

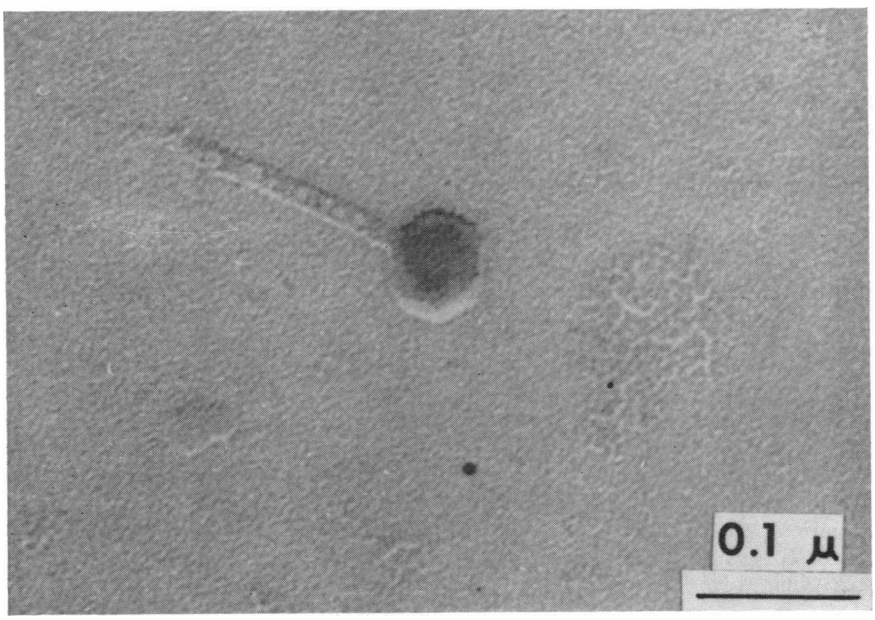

FIG. 1. *Electron micrograph of T1 phage particle prepared by air-drying.*

nucleic acid and ribonucleic acid decreased the viscosity of the suspension and enhanced the separation of the phage component by centrifugation (Herriott and Barlow, 1952). The suspension was clarified by low-speed centrifugation and then subjected to six complete cycles of differential centrifugation, i.e., alternate high-speed (60,000 × *g*) and low-speed (10,000 × *g*) centrifugation. The pellets from the high-speed centrifugation were resuspended in the bottom supernatant fluid and washed with triple-distilled water to dilute out the salts. From the original 5 liters of crude lysate, about 70 ml of aqueous purified phage titering up to 10^{12} phage particles per milliliter was produced. Loss of viable phage particles from the purification process was about 80% in most cases.

For sampling trials, several purified lots that had been stored at 5 C for periods up to 1 year were pooled, clarified at low-speed centrifugation, and put through a final cycle of differential centrifugation. The pooled suspension stored very well at 5 C; there was no discernible drop in viable titer during the period of this study, which was nearly 2 months.

The physical and biological properties of the radioactive phage suspension used in all trials were: viable count, 1.55×10^{11} phage per milliliter; radioactivity, 10 μc per milliliter; viscosity (30 C), 1.0349 centipoises; specific gravity, 1.001; surface tension, 74.4 dynes per cm; dry weight, 0.01%; *p*H, 6.6.

Radioactive tracer. A radioactive isotope was selected as the physical tracer because the minute amounts of carrier-free isotope required would not alter the characteristics of the phage suspensions and therefore would not increase the aerosol particle size. Soluble radioactive phosphate was used as an extracellular tracer, i.e., it was added directly to the phage suspension. The isotope, specially prepared for this study by the Oak Ridge National Laboratory, Oak Ridge, Tenn., was a solution of carrier-free $H_3P^{32}O_4$ in distilled water of an activity of 1 mc/ml. It had a *p*H of 6.2, which was compatible with that of the phage suspension, 6.6. The isotope solution was added to the phage suspension at the start of each test. For all tests the amounts were constant (0.1 ml of isotope solution per 9.9 ml of phage). As a consequence, the activity of the test phage suspension decreased, because of radioactive decay, from 10 μc/ml for the first test to less than 1 μc/ml for the last test, nearly 2 months later.

Preliminary experiments revealed that phage inactivation by the radioactive tracer was not detectable over a 24-hr holding period. In the sampling trials, the phage-isotope contact period, i.e., the time between sample collection and assay, was less than 4 hr.

Aerosol generator. The particle size of aerosols produced from liquid suspensions is primarily a function of the atomizer droplet size and the constituency or amount of solid material in the spray suspension. Phage aerosols of the minimal particle size would therefore be produced from clean aqueous phage suspensions with an atomizer producing the smallest droplets.

The Dautrebande $D_{30}1$ aerosol generator (J. H. Emerson Co., Cambridge, Mass.) has been used for the production of submicron aerosols from solutions of dyes and salts. It is a type of atomizer-impactor in which the larger droplets are returned to the solution, whereas the smaller droplets are permitted to escape. This selection is accomplished by means of successive liquid barriers, which is termed obligatory liquid filtration. The obligatory liquid filtration takes place in a hollow cylinder in which the unstable particles coalesce and form a continuous liquid mass. The droplets that are able to pass the barrier are passed through a capillary pipe system where particles that have not attained a sufficient degree of stability coalesce and form another liquid barrier through which only the most stable droplets can traverse. The dispersion process involves some evaporation, and consequently there is a progressive (linear) concentration of the generator suspension (Dautrebande, 1962).

Aerosol chamber. The aerosol chamber and apparatus used to test the air-sampling devices are illustrated in Fig. 2. Dynamic aerosols were produced by atomizing radioactive aqueous purified T1 phage suspensions with a Dautrebande $D_{30}1$ aerosol generator operated at 17.5 psi. The air flow through the generator was 18 liters per min and the fluid atomization rate was 0.15 ml/min. The aerosol was diluted and mixed with 30 liters of air of the desired relative humidity in a 4-liter cylindrical glass chamber. From the mixing chamber, the aerosol passed into a 12-gal carboy and finally into a circular (10 cm in diameter) manifold with 10 sampling ports. The excess aerosol was bled off through a filter located upstream from the sampling manifold. Aerosol relative humidity was controlled by dryers and humidifiers for the mixing air. Aerosol relative humidity and temperature measurements were obtained from wet and dry bulb thermometers. Air supplies for generation and mixing were filtered, which assured clear particle-free air. Standard operation involved generating the aerosol continuously for a total of 25 min (10 min to establish the desired humidity followed by a 5-min sampling period and a final 10 min to recheck the humidity). The samplers removed three-fourths of the total inflow, consisting of 48 liters per min. To minimize the radiological hazard, the system was housed in a ventilated chemical hood.

Aerosol samplers. Five aerosol samplers were evaluated in comparative tests for the physical (radioactive) and viable collection of submicron T1 phage aerosols. They included two types of high-velocity liquid impingers, two ultrahigh-efficiency filter papers, and a low-flow bubbler. Slippage of the aerosol through the samplers was determined from backup filters placed in the sampler exhaust line. The following samplers were

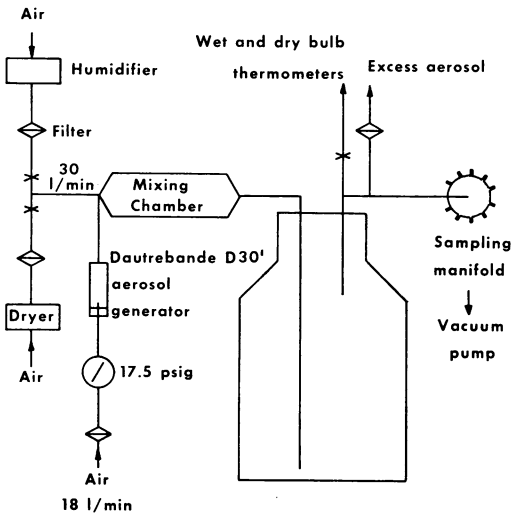

FIG. 2. *Diagram of aerosol apparatus.*

evaluated simultaneously for 5-min sampling periods.

All-glass impinger (AGI-4). This sampler is a high-velocity liquid impinger evaluated at the maximal (~ sonic) flow rate of 12.5 liters per min with 20 ml of nutrient broth, containing 0.06% Dow Corning Antifoam A (Dow Corning Corp., Midland, Mich.). The flow rate is controlled by a capillary orifice at the lower end of the inlet tube. The tip of the capillary is 4 mm from the flask bottom, hence the designation AGI-4 (Wolf et al., 1959). The raised impinger (AGI-30) was not included in this study, because preliminary tests with nonradioactive submicron T1 phage aerosols revealed that the AGI-4 was superior for viable collection at all three aerosol relative humidities.

Capillary impinger. This sampler is similar in principle to the AGI-4 except that maximal (~ sonic) flow rate is 2.5 liters/min. It was evaluated at an orifice-to-flask-bottom clearance of 8 mm with 25 ml of broth with antifoam (Wolf et al., 1959).

Fritted bubbler. This sampler is an air washer-bubbler made from a fritted-glass disc (Corning Glass Works, Corning, N.Y.) and a 250-ml flask. The fritted-glass disc at the end of the inlet tube provides for uniform distribution of the bubbles and intimate gas-liquid washing. The sampler was operated at a low flow rate of 1.0 liter per min to enhance collection by diffusion. The flow was controlled by a capillary orifice in the vacuum line. The fritted-glass disc, 20 mm in diameter and of coarse porosity with a maximal pore size of 40 to 60 μ, was immersed in 150 ml of broth with antifoam.

Chemical Corps Type 6 filter paper. This material is an ultrahigh-efficiency filter paper composed of cellulose, rope, and asbestos fibers (Decker et al., 1962). The filter discs (2.5 cm in diameter) were sealed in in-line filter holders with an effective area of 2.0 cm^2 (Wolf et al., 1959). Sampler flow rate was 1.0 liter per min, and therefore the filter face velocity was 8 cm/sec. The flow rate was controlled by a capillary orifice placed in the vacuum line. For assay, the filter discs were placed, immediately after the test, in 100 ml of broth containing 0.1% Tween 20 (Atlas Powder Co., Wilmington, Del.) and were shaken vigorously for 10 min on a mechanical shaker. The Type 6 paper is easily disintegrated to pulp by shaking.

Glass filter paper. MSA 1106BH glass filter paper (Mine Safety Appliances, Pittsburgh, Pa.) is an ultrahigh-efficiency filter paper composed of very finely spun glass fibers and an organic binder to increase tensile strength. Unlike Type 6 paper, it does not break up with shaking.

Backup filter. The sampler slippage was determined from in-line filter holders containing Type 6 filter paper backed up in the same holder by Chemical Corps Type 5 filter paper. The Type 5 is a low-efficiency paper of cellulose and asbestos fibers with a backing of cotton scrim. It served as a support to prevent rupture of the Type 6 paper and was assayed with the Type 6 paper.

The toxicity of the filter papers to phage and *E. coli* was investigated. None of the three filter papers is toxic to phage, but Type 6 paper inhibits the growth of *E. coli*, which causes a reduction in phage plaque counts. It exhibited inhibition, presumably a bacteriostatic effect, only when the filters were suspended in small volumes of broth and plated without dilution. Inhibition was demonstrated by suspending Type 6 filter discs in 20, 50, and 100 ml of Tween broth of a known phage concentration. Mean phage counts from 1-ml samples of the suspensions were 68% of controls for 20 ml, 87% for 50 ml, and 100% for 100 ml. In the sampling trials, 100-ml blanks were used.

Sampler air-flow rates were determined with a wet test meter. They were calibrated as they were to be used in the tests, i.e., with backup filters. The increase in resistance due to the backup filter reduced the AGI-4 flow rate to 95% of the maximal (~ sonic) flow. For the capillary impinger, this value was 99%. The effect of this deviation from sonic velocity on impinger performance for submicron particles was not determined.

In the tests, the samplers were attached to a vacuum manifold. The absolute pressure at the manifold was less than 0.4 atmosphere, sufficient to maintain pressure drops exceeding 0.5 atmosphere across the sampler orifices and assuring constant maximal flows.

Aerosol particle sizing. Preliminary tests of nonradioactive T1 phage aerosols with the Casella Cascade Impactor (Sonkin, 1950) and the Andersen Sampler (Andersen, 1958), both of which are used for sizing viable microbial aerosols in the micron size range, revealed that T1 phage aerosols were somewhere in the submicron size range, but no finite parameters could be calculated because

more than 90% of the viable particles passed through these samplers. Solid gelatin, after the work of Dahlgren, Decker, and Harstad (1961), was the collecting medium used in the Andersen sampler. Finite particle size parameters of the radioactive T1 phage aerosols were determined with an electron microscope from aerosol samples collected with an electrostatic precipitator. Electrostatic precipitation is the sampling method of choice for sizing aerosols below the limits of resolution of the optical microscope (Fraser, 1956). The precipitator used in these tests is similar to those described by Billings and Silverman (1962) and Morrow and Mercer (1964) and consists of a glass tube (1.75 cm in diameter) holding two electrodes. One electrode is a needle. The opposing electrode is a brass holder for an electron microscope specimen grid. A potential of 10,000 v a-c is maintained across the electrodes and the distance between the electrodes is adjusted so that a corona is barely visible. This distance was about 1.8 cm for my instrument. An air-flow rate of 1.0 liter per min was used, giving a linear velocity through the precipitator of 7 cm/sec. The specimen grids were collodion-coated and shadowed with carbon. A sampling time of 5 min was usually required to collect a sufficient number of particles on the grid. The grids were then shadowed with uranium and examined in the electron microscope at a magnification of 6,900. Random areas from grids with a particle population of 25 to 100 particles per field were photographed. For sizing, the electron micrographs were projected on a screen of translucent paper to a total magnification of 50,000 (1 mm = 20 mμ). All particles were measured. It was not possible to distinguish phage-bearing particles from empty particles; therefore only the physical or total aerosol particle size was determined. The individual particles were measured according to Martin's statistical diameter to the nearest 0.5 mm. Martin's statistical diameter has the effect of avoiding bias in the measurement of irregular-shaped particles. It is defined as the mean length of a line intercept by the profile boundary that approximately bisects the area of the profile. The bisecting line is taken parallel to a fixed direction, irrespective of the orientation of each particle (Herdan, 1953). For each size interval, the number of particles, the percentage of the total number of particles, and the cumulative percentages were determined. The cumulative percentages, when plotted against particle diameter, gave a good straight-line fit on log probability paper. Therefore, the particle size distribution was log-normal. Populations of 200 to 500 particles were used to determine the following parameters: number median diameter (NMD), mass median diameter (MMD), geometric standard deviation (GSD), per cent by number less than 60 mμ in diameter, per cent by mass less than 60 mμ in diameter. The geometric standard deviation, which indicates the degree of heterogeneity of the aerosol, was determined from the following equation:

$$\mathrm{GSD} = \frac{84.13\%\ \text{size}}{50\%\ \text{size}} = \frac{50\%\ \text{size}}{15.87\%\ \text{size}}$$

The MMD was calculated from the NMD graphs by using the following equation derived by Hatch (1933), which is valid only if the distribution is log-normal:

$$\log \mathrm{MMD} = \log \mathrm{NMD} + 6.9078 \log^2 \mathrm{GSD}$$

Assay. Aerosol samplers and phage suspensions were assayed in duplicate, both for radioactivity and for T1 phage. Dilution for the phage assays reduced the soluble tracer to negligible amounts, terminating the exposure of phage to radiation. Samples were assayed for phage by making duplicate serial dilutions in nutrient broth and plating 1-ml samples of each dilution in triplicate, by use of an agar layer method similar to that described by Adams (1959). Fresh nutrient broth cultures of *E. coli*, aerated on a shaking machine at 37 C for 4 to 6 hr, were the seed cultures. Test tubes containing 3 ml of melted dilute nutrient agar (11 g per liter) were inoculated with 0.5 ml of the seed culture, and 1 ml of the phage dilution was added. The mixture was swirled briefly and poured over the surface of a solid nutrient agar plate. The plate was rocked gently and allowed to harden. The melted dilute agar tubes were held at 46 C in a water bath prior to seeding with the *E. coli* culture. The phage plaques were counted after incubation at 37 C for 5 hr.

For the radioactive P^{32} assays, duplicate 2-ml samples were pipetted into aluminum planchets, evaporated to dryness overnight, and counted the next day in a windowless gas-flow proportional counter of 50% geometry. A strong attempt was made to keep the method of planchet preparation constant. To compare the results of the trials, the activity of the samples at zero-time was computed from the observed counts by using the decay curve of P^{32} (half life = 14.3 days). Zero-time was selected as the time when the specific (absolute) activity of the isotope solution, which was added to the phage suspension at the start of each trial, was 1.0 mc/ml. The specific activity of the samples was determined by calibrating the proportional counter with a known standard and making the appropriate correction for back-scatter. Self-absorption was negligible.

Design of experiment. A series of 15 aerosol trials was conducted to compare five air-sampling devices for the physical and viable collection of radioactive submicron T1 phage aerosols at relative humidities of 30, 55, and 85% and a temperature of 24 C. Five trials were conducted at each relative humidity, with use of duplicate samplers of each type per trial.

RESULTS

Data from 15 aerosol trials showing the tracer and phage concentrations in the Dautrebande

TABLE 1. *Effect of aerosolization and sampling on the viability of T1 phage*

Aerosolization	Time	Radioactivity		Phage count		Viability	
	min	*μc/ml*		*× 10¹⁰/ml*		%	
Dautrebande $D_{30}1$ aerosol generator[a]	0	10		15.5		100	
	12.5	12		10.0		54	
	25	14		6.3		29	
		× 10⁻³ μc/liter		*× 10⁵ phage/liter*			
Aerosol chamber							
Theoretical[b]	12.5	16.6		138		No basis for determining	
Effective[c]	12.5	6.3		52.5			
Test sampler[d]	Relative humidity	Samplers		Samplers		Samplers	
		Test	Backup[e]	Test	Backup	Test	Backup[e]
	%	(× 10^{-3} μc/liter)		(× 10^6 phage/liter)		%	%
All-glass impinger (AGI-4)	30	3.3	3.0	2.3	0.73	8.4	2.9
	55	4.0	2.3	2.9	0.55	8.6	2.7
	85	4.4	1.9	0.89	0.30	2.4	1.9
Capillary impinger	30	3.3	3.0	2.3	0.51	8.4	2.0
	55	3.4	2.9	2.8	0.50	9.8	2.1
	85	3.8	2.5	0.87	0.34	2.7	1.6
Type 6 filter paper	30	6.3	(0.001)	1.2	0.0006	2.3	(5)
	55	6.3	(0.001)	2.1	0.0013	4.1	(10)
	85	6.3	(0.004)	0.70	0.0006	1.3	(2)
1106BH filter paper	30	6.0	(<0.0006)	0.23	0.0006	0.5	(>10)
	55	6.0	(<0.0006)	0.34	0.0003	0.7	(>5)
	85	6.0	(0.002)	0.27	0.0003	0.5	(2)
Fritted bubbler (coarse porosity)	30	1.2	5.1	0.56	0.44	5.6	1.0
	55	1.2	5.1	0.51	0.42	5.1	1.0
	85	1.1	5.2	0.28	0.40	2.9	0.9

[a] Means of 15 aerosol trials.

[b] Based on radioactive and phage counts of Dautrebande suspension at 12.5 min, flow rates of suspension and air, and chamber volume.

[c] Based on the actual total (test + backup) radioactive recoveries of all samplers, except 1106BH, from the 15 trials.

[d] Means of five trials with two samplers per trial.

[e] Numbers in parentheses give approximate values based on additional tests of Table 3 that were not assayed for phage.

suspension and in the test and backup samplers are summarized in Table 1. From these data, the following parameters were calculated and are listed in Tables 1, 2, and 3.

Killing of phage by the Dautrebande generator. There was considerable destruction (death) of phage by the Dautrebande $D_{30}1$ aerosol generator (Table 1). Radiological and phage assays of Dautrebande suspensions at intervals during the 25-min aerosolization period revealed an exponential decrease in phage titer and a linear increase in radiological titer. The exponential form of the equation that describes the rate of phage inactivation in the Dautrebande suspension is

$$\frac{a}{x} = \frac{a_0\, e^{-t/T}}{x_0}$$

where a is the phage count at any time t, a_0 is the original count at zero-time, x is the radioactivity at time t, and x_0 is the original radioactivity at zero-time. Viability, i.e., the fraction of phage surviving in the Dautrebande suspension at any time, is ax_0/a_0x. At the midpoint of the aerosol sampling period (t = 12.5 min), 54% of the phage particles that were viable at zero-time were still viable.

Radiological aerosol concentrations. The Dautrebande generator concentrated the tracer in a

TABLE 2. *Comparison of air-sampling devices for the viable and physical collection of radioactive submicron T1 phage aerosols at 30, 55, and 85% relative humidity (RH)**

Test sampler	Flow rate	RH	Relative viable efficiency†	Physical slippage	Viable slippage
	liters/min	%	%	%	%
All-glass impinger (AGI-4)	~Sonic	30	100	48	24
	12.5	55	100	37	16
		85	100	30	25
Capillary impinger	~Sonic	30	100	48	18
	2.5	55	97	46	15
		85	98	40	28
Type 6 filter paper	1.0	30	52	—‡	0.05
		55	75		0.06
		85	79		0.09
1106BH filter paper	1.0	30	10	—	0.26
		55	12		0.09
		85	30		0.12
Fritted bubbler (coarse porosity)	1.0	30	24	81	44
		55	18	81	45
		85	31	82	59

* Each value represents mean of 10 samplers.

† AGI-4 as the reference sampler: test sampler/AGI-4 × 100. Mean AGI-4 recoveries: 30% RH, 2.3×10^6 phage/liter; 55% RH, 2.9×10^6 phage/liter; 85% RH, 8.9×10^5 phage/liter.

‡ Backup filter recoveries indistinguishable from background radiation.

TABLE 3. *Slippage of radioactive submicron T1 phage aerosols through Type 6 and 1106BH filter papers at 30, 55, and 85% relative humidity (RH)**

Test filter†	Flow rate	RH	Physical slippage
	liters/min	%	%
Type 6	1.0	30	0.02
		55	0.02
		85	0.07
1106BH	1.0	30	<0.01
		55	<0.01
		85	0.03

* Each value represents mean of five samplers.

† Test filter activity: 7×10^5 counts/min .

linear manner, and therefore the radiological aerosol concentrations increased with time (Table 1). The mean theoretical radiological concentration at the midpoint of the sampling period (t = 12.5 min) was 16.6×10^{-3} μc per liter. It was derived from the radioactive counts of the Dautrebande suspensions at 12.5 min, fluid atomization rate, air-flow rates for generation and mixing, and aerosol chamber volume. The effective radiological aerosol concentration was a mean of the actual total sampler recoveries. Total sampler (test + backup) recovery averaged 6.3×10^{-3} μc/liter for the five trials at each of the three relative humidities. Therefore, about 60% of the aerosol was physically lost on passage through the apparatus.

Phage aerosol concentrations. The phage concentrations (Table 1) in the aerosol chamber were calculated from the radiological aerosol concentrations and the ratio of phage to tracer a/x in the Dautrebande suspension at the midpoint of the sampling period (t = 12.5 min) according to the formula $X(a/x)$ where X is the radiological aerosol concentration (theoretical or effective) and a/x is the ratio of phage to tracer. There was no basis for determining the viability of the phage aerosol. Phage aerosols are subject to biological decay (death); therefore, the number of living phage particles expected to be available in the aerosol for sampling was unknown, but must be less than 52.5×10^6 per liter.

Killing of phage by the samplers. There was considerable destruction of phage by the samplers (Table 1). Viability refers to the fraction of phage collected by the samplers that remains viable. It is a measure of the destructiveness of the samplers for T1 phage and was calculated from the ratio of phage to tracer in the sampler and the ratio of phage to tracer in the Dautrebande suspension at t = 12.5 min:

$$\text{viability} = \frac{\dfrac{\text{sampler phage count}}{\text{sampler radioactivity}}}{\dfrac{\text{Dautrebande phage count}}{\text{Dautrebande radioactivity}}}$$

Obviously, this did not compensate for loss of the phage aerosol due to biological decay (death), but gives a basis for comparing sampler kill. The high-velocity impingers, AGI-4 and capillary impinger, were the least destructive, followed by fritted bubblers, Type 6 filter paper, and 1106BH filter paper. Sampler kill was highest at 85% relative humidity and lowest at 55% relative humidity. The 1106BH filter paper was much more destructive than Type 6 filter paper.

Relative viable sampler collection efficiency. Relative viable collection efficiency (Table 2) was based on the phage assay of test samplers and expressed as a percentage of the reference sampler, AGI-4, as given by:

$$\text{relative viable efficiency} = \frac{\text{test sampler}}{\text{AGI-4}}$$

The high-velocity impingers, AGI-4 and capillary impinger, gave the highest viable recoveries, followed by Type 6 filter paper, fritted bubblers, and 1106BH filter paper. Both types of filter paper improved with an increase in aerosol relative humidity, but Type 6 was superior to 1106BH paper for viable collection. Viable recoveries were highest at 55% relative humidity and lowest at 85% relative humidity.

Sampler physical slippage. Physical slippage (Table 2) was based on the radiological assay of test samplers and backup filters as given by:

$$\text{physical slippage} = \frac{\text{backup filter}}{\text{backup filter} + \text{test sampler}}$$

The high-velocity impingers, which are widely used for micron-size aerosols, such as bacteria, where slippage is less than 1%, were not efficient physical collectors of submicron particles. Impinger slippage decreased with an increase in aerosol relative humidity, presumably because of the change in aerosol particle size with relative humidity. However, the differences in particle size at the three aerosol relative humidities were small. The fritted bubbler, a sampler designed to collect particles by the diffusion principle, was the poorest submicron sampler in that physical slippage was greater than 80%.

Sampler viable slippage. Viable slippage (Table 2) was based on the phage assay of test samplers and backup filters as given by:

$$\text{viable slippage} = \frac{\text{backup filter}}{\text{backup filter} + \text{test sampler}}$$

As expected, viable slippage was less than physical slippage because backup filters (Type 6 paper) as well as the test samplers themselves are destructive to phage. For both types of impingers, viable slippage was lowest at 55% relative humidity. The viable slippage parameter, although it is an obvious way of presenting the data, is not very useful; the other parameters are more meaningful in describing sampler performance.

Filter paper physical slippage. The filter papers were the most efficient samplers for the physical collection of submicron aerosols, but finite slippage values were not obtained because backup recoveries were indistinguishable from background radiation (Table 2). Additional tests, shown in Table 3, were conducted by use of much higher effective radiological concentrations in an attempt to obtain finite values. The filter papers were not assayed for phage in these tests. The effective aerosol concentration was increased 120 times by increasing the sampling period to 150 min and suspending the filters in a smaller volume (25 ml) of fluid, but again backup recoveries were low. One aerosol trial was conducted at each relative humidity, with use of five samplers of each filter paper per trial. Finite slippage values are shown only where backup filter recoveries were significantly above background. Both filter papers were very efficient for submicron particles, but 1106BH was superior to Type 6 paper for physical collection. Slippage was highest for both papers at 85% relative humidity.

Not all the radioactivity was removed from the 1106BH filters by shaking. The 1106BH paper does not disintegrate into pulp as does Type 6 paper, and recoveries were slightly lower than the total or actual radioactivity. This was demonstrated by centrifugation techniques involving the analysis of the supernatant fluid and sediment and in the sampling trials (Table 1), where 1106BH filter paper recoveries were 95% of Type 6 recoveries which, by the same techniques, were shown to be representative of the total activity.

Aerosol particle size. Aerosol particle size was adequately described by a log-normal distribution. The particle size parameters of radioactive T1 phage aerosols at 30, 55, and 85% relative humidity are shown in Table 4. Typical results are shown in Fig. 3 and 4. Figure 3 is a graph of the 55% relative humidity aerosol of Day I, showing the cumulative percentages by number and by mass plotted against particle diameter on log probability paper. The median diameters (50% intercepts) are 119 mμ NMD and 172 mμ MMD. Figure 4 is an electron micrograph of this aerosol. Differences between the particle size parameters at the three relative humidities were small, but the particles were slightly larger at 85% relative humidity.

A radioactive tracer was used in this study to

follow both the physical (radioactive) and viable presence of the aerosol particles. However, there was a deviation from this concept, because, whereas all the aerosol particles were radioactive, a small percentage of the aerosol particles were smaller than the size of a single phage particle. In sizing the aerosols from electron micrographs, it was not possible to distinguish phage-bearing particles from empty particles, but all particles were measured. Therefore, only the total or physical aerosol particle size was determined. Phage assays are a function of the number of particles, and radiological assays are a function of mass. Inspection of the NMD graphs reveals that less than 5% of the aerosol particles were smaller than a single phage particle (60 mμ in diameter). Therefore, many of the aerosol particles contained more than one phage particle, and disintegration of phage clumps by the samplers could be a possible cause of sampler differences for viable collection. However, not all of the aerosol particles larger than 60 mμ contained phage, because aerosol particle size is related to the amount of solid material in the Dautrebande suspension, and it cannot be assumed that all of the extraneous material was removed from the phage suspensions by the purification process. The 60-mμ intercept of the MMD graphs was less than 0.2%; i.e., the fraction of the total aerosol radioactivity associated with the known empty particles was less than 0.2%.

TABLE 4. *Particle size parameters of radioactive T1 phage aerosols at 30, 55, and 85% relative humidity (RH)*

RH	Day	No. of particles	Median diam		Less than 60 mμ diam		Geometric SD
			No.	Mass	No.	Mass	
%			mμ	mμ	%	%	
30	I	219	126	167	1	0.04	1.36
	II	382	114	181	5	0.2	1.48
55	I	485	119	172	2	0.1	1.42
	II	387	117	186	4	0.2	1.48
85	I	225	128	194	2	0.08	1.45
	II	272	132	209	2	0.06	1.48

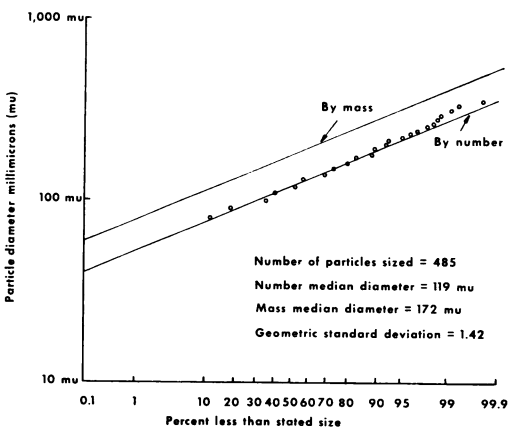

FIG. 3. *Size distribution of radioactive T1 phage aerosol at 55% relative humidity dispersed with Dautrebande $D_{30}I$ aerosol generator.*

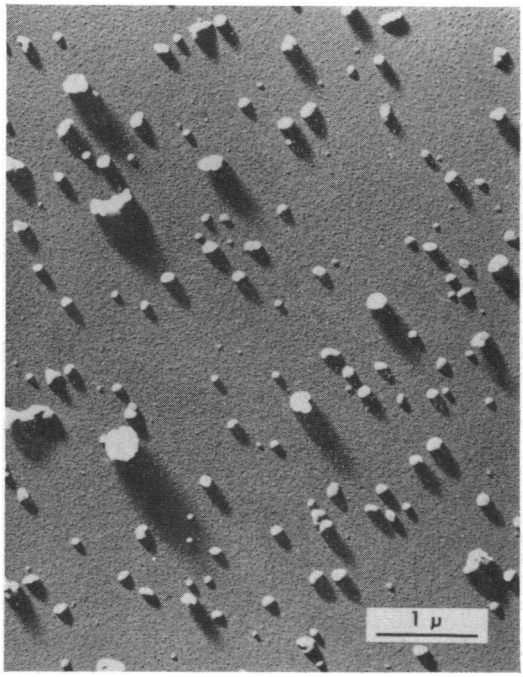

FIG. 4. *Electron micrograph of radioactive T1 phage aerosol at 55% relative humidity dispersed with Dautrebande $D_{30}I$ aerosol generator.*

DISCUSSION

All of the samplers tested were partial collectors of viable submicron phage aerosols. Viable collection, unlike physical slippage, is specific for T1 phage and is a function of both the physical and biological properties of the aerosol, Sampler differences for viable collection were attributed to incomplete physical collection (slippage) and destruction (death) during the sampling process. Other possible causes believed to be of less importance are disintegration of clumps by the sampler and retention in the sampler, i.e., inability to remove particles completely from 1106BH filter paper. Physical slippage and dis-

integration are related to the aerosol particle size. Destruction may also be a function of particle size. Physical slippage was quantitated, but it was not possible to distinguish between destruction and disintegration because (i) phage aerosols are subject to biological decay, and therefore the viable aerosol concentration at the moment of sampling was unknown; and (ii) radiological assays are a function of mass and not of the number of particles; they do not reflect breakup of clumps. However, there was strong but indirect evidence of phage destruction by the samplers, and phage inactivation by the Dautrebande generator was proved. The high-velocity impingers, AGI-4 and capillary impinger, gave the highest viable recoveries but allowed considerable slippage. Impinger physical slippage varies inversely with relative humidity, presumably because of changes in particle size with relative humidity. However, the particle size differences at the three relative humidities were small, indicating that impinger slippage is extremely sensitive to changes in the size of submicron particles. Type 6 filter paper is virtually a complete physical collector of submicron particles, but cannot, in a strict sense, be classified as a representative sampler, because destruction may be a function of aerosol particle size.

ACKNOWLEDGMENTS

I wish to thank Robert Sine and Billy Paseur for electron microscopy, Roger Scherff for advice and the use of radiological facilities, Joseph Milo, John Happ, and Carl Michael for skilled technical assistance, and M. B. Albert for derivation of the theoretical radiological aerosol concentrations.

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