J. Hyg., Camb. (1960), **58**, 373
Printed in Great Britain

Measurement of the physical decay of aerosols by a light scatter method compared to a radioactive tracer method*

By ROBERT L. DIMMICK

Naval Biological Laboratory, School of Public Health, University of California, Berkeley, California

(*Received* 24 *June* 1960)

INTRODUCTION

The necessity for distinguishing between loss attributable to physical factors (physical decay) and loss of viability (biological decay) in bacterial aerosols examined in unventilated settling chambers has been discussed recently by Harper, Hood & Morton (1958). They suggested that organisms labelled with ^{32}P should be included in the atomizer fluid to act as tracers of the loss of particulate matter. Light scatter methods, however, have been employed routinely in this laboratory to determine the particle size of airborne matter (Dimmick, Hatch & Ng, 1958) and to provide a relative measurement of physical decay. The rate of physical decay is then used to adjust the rate of combined physical and biological loss (total decay) as measured by counting the number of viable cells remaining airborne.

In the study to be described, the physical fall-out of atomized aerosols has been determined in two types of aerosol chambers by both the tracer and light scatter methods. The two methods appear to be equivalent, providing a suitable analysis of the decay curves is employed.

MATERIALS AND METHODS

Serratia marcescens (strain 8UK) was grown in synthetic medium (Bunting, 1940) with the usual source of phosphorus replaced *in toto* by radioactive sodium phosphate (^{32}P) at a level of 150 mC./l. of medium. After the culture had incubated for 24 hr. at 34° C., an aliquot was removed and atomized immediately. The remainder of the culture was centrifuged, and the supernatant fluid was recovered and stored at 4° C. for subsequent use. The centrifuged cells were then washed twice in Bunting's medium and atomized from this menstrum.

Organisms from a second culture of *S. marcescens*, grown on non-radioactive Bunting's medium, were washed by centrifugation and suspended in the radioactive supernatant fluid from the first culture. It was then possible to compare all combinations of radioactive and non-radioactive preparations in the aerosolized state.

The suspensions of bacteria were sprayed from a Wells-type refluxing atomizer into a stirred settling chamber (1500 l. capacity) similar to that described by

* This work was sponsored by the U.S. Army Chemical Corps, Fort Detrick, Frederick, Md., and the Office of Naval Research, United States Navy, under a contract between the Office of Naval Research and the Regents of the University of California.

Dimmick *et al.* (1958). A suspension of the radioactive cells in radioactive medium was also atomized into four rotating drums of 1500 l. capacity (Goldberg, Watkins, Boerke & Chatigny, 1958). The spray time varied from 2 to 3 min. The suspensions contained about 10^9 viable bacteria per ml.

Two types of impingers were employed to sample the particulate content in the rotating drum experiments. One was an all glass raised impinger, similar to that used by Harper *et al.* (1958), containing 20 ml. of distilled water with 0·01 % Dow Corning anti-foam B and was operated at a flow rate of 12·5 l./min. The other was a submerged capillary inserted in an 8-oz. French square prescription bottle and was the only sampler employed in the settling chamber experiments. This impinger used 20 ml. of the fluid described above and was operated at a flow rate of 6·7 l./min.

After samples had been taken, 2 ml. of the impinger fluid were transferred to aluminium planchets. The fluid on the planchet was evaporated to dryness and the level of radioactivity was measured with a Tracerlab thin-window counter. The activity was expressed as counts per sec. (CPS) per l. of aerosol and these counts, adjusted to percentage of the first sample, were plotted versus time of sampling on semi-log paper to permit comparison with the light scatter decay curve. The radioactivity per ml. of the atomized fluid was determined in the same way to permit calculation of the initial physical recovery of aerosol.

Instruments employed to determine decay by light scatter measurements (Dimmick *et al.* 1958) were composed essentially of 931-A photomultiplier valves activated by light scattered from particles as they passed through a light beam. The monitored area was about 4 cm.^{2} The particles passed through this area constantly and rapidly. Thus, the total scattered light measured was an average value not influenced significantly by the light scattered from a single particle and is referred to as the light scatter area. The meter which recorded photomultiplier response was set to 'zero' before atomization and the values thereafter were read directly from the instrument meter which had previously been shown to respond linearly to changes in light intensity.

In the settling chamber the decay of the light scatter area was recorded continuously, whereas in the drums the values were determined only during the sampling periods. In both instances values were plotted on semi-log paper versus linear time to obtain a measure of the physical decay pattern.

RESULTS

The initial recovery of particulate matter (Table 1) from the settling chamber, based on a fluid output of 0·25 ml./min., was 33–41 %, except for that experiment wherein the bacteria alone contained radioactivity. This was caused in part by particulate loss within an inlet pipe through which the aerosol was conducted before samples were taken.

Decay curves are presented in Figs. 1 and 2 to permit a visual comparison of the similarity between data obtained from scattered light measurements and from radioactivity measurements. It is evident that during the first 50–100 min. of aersol time the decay patterns were not exponential and that radioactive airborne

matter was decreasing faster than might be expected from the light scatter curves. After the initial fall-out of particles the slopes were similarly exponential for both methods of measurement. Use of the term exponential herein refers to decay which exhibits a straight-line relationship on semi-log paper and is obviously associated with the latter portion of the aerosol decay patterns.

Table 1. *Initial recovery and half-life of* ^{32}P *labelled aerosols*

Suspension		Theoretical	Observed	Per	Half-life of exponential decay	
Labelled* portion	CPS†/ml.	CPS/l. aerosol	CPS/l. aerosol	cent recovery	Light scatter	^{32}P
Cells and medium	$2{\cdot}80 \times 10^5$	$8{\cdot}40 \times 10^2$	$3{\cdot}45 \times 10^2$	41	300	270
Medium	$2{\cdot}48 \times 10^5$	$6{\cdot}60 \times 10^2$	$2{\cdot}18 \times 10^2$	33	366	366
Medium	$2{\cdot}48 \times 10^5$	$5{\cdot}80 \times 10^2$	$2{\cdot}33 \times 10^2$	38·4	300	240
Cells	*$4{\cdot}24 \times 10^5$*	*$4{\cdot}24 \times 10^2$*	*$6{\cdot}00 \times 10$*	*14·2*	*270*	*210*
Cells and medium‡	$15{\cdot}0 \times 10^5$	$5{\cdot}0 \times 10^2$	$2{\cdot}98 \times 10^2$§	5·9	1600	1600

* Cells and medium were employed in all cases, but only those portions listed contained radioactivity.

† CPS = counts per second.

‡ Mean values for four aerosols dispersed in the drums. The other aerosols were dispersed in settling chambers.

§ By extrapolation to zero aerosol time.

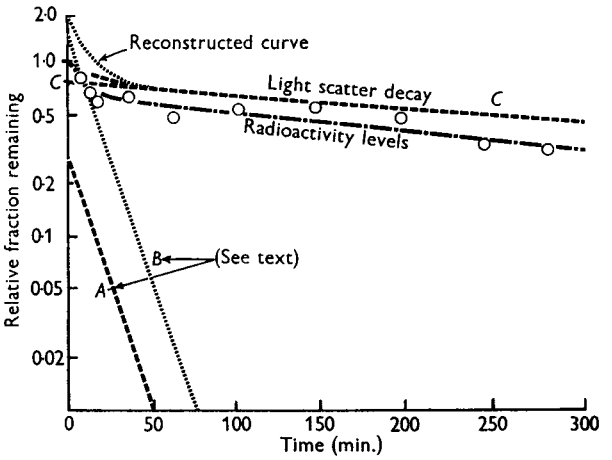

Fig. 1. Decay curve of an aerosol measured by scattered light and by the count of residual radioactivity. Only the medium contained radioactivity. The reconstructed light scatter decay curve, as described in the text, is included.

Experiments wherein the light scatter recording unit and the stirring fan of the settling chamber were operated overnight showed that the exponential decay continued to the limit of the measuring instrument's sensitivity. The lack of evidence of decreased decay indicates that there was no appreciable concentration of submicron particles present in the aerosol. The slopes of the fall-out patterns showed the size of the smallest particles to be 1·05 μ in diameter (Dimmick *et al.* 1958).

https://doi.org/10.1017/S0022172400038511 Published online by Cambridge University Press

The precision of measurement of the radioactive particles remaining airborne within the drums is shown in Table 2. The level of activity in triplicate or quadruplicate samples taken at the specified times after aerosolization varied to an extent that differences in levels between sampling time were not evident within

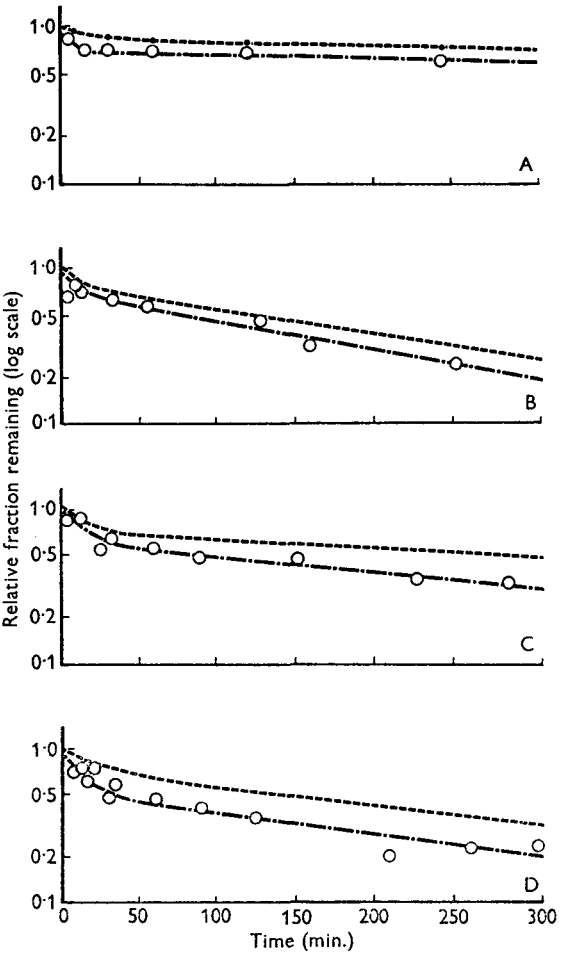

Fig. 2. Decay curves of aerosols measured by scattered light and by the count of residual radioactivity. A = Mean of four experiments in the rotating drum chamber. Both bacteria and suspending medium contained radioactivity. B = One experiment in the stirred settling chamber. Both bacteria and suspending medium contained radioactivity. C = One experiment in the stirred settling chamber. Only the medium contained radioactivity. D = One experiment in the stirred settling chamber. Only the bacteria contained radioactivity. - - - - Light scatter decay; --O-- radioactivity levels (line of best fit).

individual experiments. The average of the four trials, however, produced the decay curve shown in Fig. 2A. Table 2 also includes the light scatter values noted at the specified times. These indicate the presence of a slow decay rate within individual experiments. The table also illustrates that there was no significant difference between the sampling capabilities of the two impingers employed.

https://doi.org/10.1017/S0022172400038511 Published online by Cambridge University Press

DISCUSSION

There was no significant difference ($p > 0{\cdot}1$) between the exponential portions of the physical decay curves as determined by light scatter methods or by sampling the residual radioactivity. During the early period of aerosol life, however, the largest particles disappeared from the aerosol at constantly decreasing rates. As expected, the decay of light scatter area during the early period was not equivalent

Table 2. *Light scatter measurements and activity levels of* ^{32}P *observed in impinger samples taken from aerosols contained in* 1500 *litre rotating drums*

Aerosol time (min.)		Drum I			Drum II			Drum III			Drum IV		
		A	B	C	A	B	C	A	B	C	A	B	C
3		.	13·2	55	.	18·0	34	.	17·9	54	.	16·7	41
		.	.	57	.	.	29	.	.	.	.	.	.
15		11·3	9·0	54	16·0	18·0	25	11·2	14·3	46	18·2	15·5	41
		11·1	12·3	46	12·9	20·1	22	15·0	13·6	43	18·0	19·2	38
		13·5	14·2	.	14·0	14·1	.	10·0	14·6	.	16·9	15·6	.
		14·7	.	.	12·0	.	.	15·5	.	.	15·5	.	.
	Mean	12·6	11·8	.	13·7	17·4	.	12·9	14·2	.	17·1	16·7	.
30		.	14·0	48	.	15·8	23	.	14·5	.	.	16·6	37
		.	.	46	.	.	22	.	.	.	.	.	37
60		12·3	11·2	40	13·7	15·0	23	14·6	14·0	41	14·9	16·1	.
		22·5	15·5	44	13·8	16·6	21	15·2	18·0	40	14·8	15·1	.
		6·8	14·9	.	13·7	15·8	.	14·1	15·6	.	17·5	18·5	.
		.	.	.	17·1	.	.	11·9	.	.	15·8	.	.
	Mean	13·8	13·8	.	14·6	15·8	.	13·9	15·8	.	15·7	16·5	.
120		13·2	14·6	38	15·7	14·8	20	10·8	12·2	38	15·7	17·6	37
		12·0	.	40	11·9	.	20	10·7	.	38	12·2	.	.
		11·5	.	.	12·0	.	.	11·2	.	.	16·3	.	.
		11·7	.	.	13·0	.	.	13·3	.	.	17·2	.	.
	Mean	12·1	.	.	13·1	.	.	11·5	.	.	15·3	.	.
240		.	11·3	37	.	14·0	20	.	12·0	39	.	17·0	36
		.	12·5	37	.	14·4	18·5	.	12·0	36	.	16·0	.
		.	12·1	.	.	12·3	.	.	14·3	.	.	14·3	.
	Mean	.	11·9	.	.	13·5	.	.	12·7	.	.	15·7	.

A = Recovery in an impinger employing 8-oz. French square medicine bottles. Values are counts per sec. in 10% of the fluid used to sample 6·7 l. of aerosol. Flow rate was 6·7 l/min.

B = Recovery in an all-glass impinger. Values are counts per sec. in 10% of the fluid used to sample 6·7 l. of aerosol. Flow rate was 12·5 l./min.

C = Relative light scatter area recorded as meter readings.

to the rate of decay of radioactivity. In the former, the positions of the rates of fall of given particle diameter ranges are related as a function of r^2 (area) and in the latter as a function of r^3 (mass or volume).

A satisfactory approximation of the mass decay can be constructed from the light scatter decay curve by subtracting the exponential portion from the overall curve to obtain, for example, line *A* in Fig. 1. This line represents the mean decay

https://doi.org/10.1017/S0022172400038511 Published online by Cambridge University Press

rate of those particles larger than the ones associated with the exponential portion of the curve. If the spread of particle diameters is greater than indicated herein, multiple subtraction steps may be required. The fractions present at zero time are in relationship to each other as a function of the area. From the rate of decay the average diameters may be determined by Stokes's law or by the use of a nomograph (Dimmick *et al.* 1958). The light scatter values at zero time (25 for line A and 70 for line C in the example) are multiplied by the respective diameters (5·5 μ and 1 μ) to obtain new zero values related according to the mass. New lines are drawn from these points parallel to the original slopes. Line C, in this example, did not change since the associated diameter was 1 μ; line A transformed to line B. Addition of the new lines at appropriate time intervals yields the mass decay. In the example, the approximate mass decay is shown by the upper dotted line which corresponds to the mass decay as determined by radioactivity measurements.

Data of a precise nature concerning biological decay have not been included in this paper for two reasons. First, the biological decay of non-sporulating organisms varies considerably more with other factors such as temperature, ambient humidity and suspending medium (Kethley, Fincher & Cown, 1957) than it does with particle size and, therefore, has little relationship to comparative measures of physical losses. Secondly, as Harper *et al.* (1958) have pointed out, the presence of radioactivity within the cell may influence its biological behaviour. This was evidenced by the loss of viability, after 15 min. of aerosol time, only in clouds generated from suspensions of radioactive bacteria.

That there was no appreciable mass composed of particles which are smaller than the bacterium plus the surrounding medium was shown by the experiment wherein only the medium was radioactive, and by the experiments showing a continuous exponential slope. If electrostatic, diffusive forces or changes in humidity were influential in causing a significant increase in decay rates, an inverted pattern of decay should have appeared. As a result, there is no evidence which would contra-indicate the method suggested by Harper *et al.* (1958) that soluble radioactive phosphate be included in the medium as a physical tracer of aerosolized virus.

Both the light scatter method and the tracer method appear to be useful, but each possesses certain advantages and disadvantages. Theoretically, the tracer method is the more direct and, because it yields absolute values, can be employed to determine recovery percentages and collection efficiencies upon initial aero-solization. It is expensive in terms of time, however, both in the sense of man-hours and of the waiting period before physical behaviour data become available for analysis. For routine use the method could be made more accurate than the presently presented data indicate.

The light scatter decay method yields data in terms of rates, and although some of the rates are approximations (as illustrated in Fig. 1), a sensitive measure of aerosol presence and behaviour is constantly available. The calculation of the physical decay curve requires only a few minutes, and aerosol behaviour is not disturbed since samples are not removed from the aerosol chamber. Recording the aerosol build-up and decay in aerosol chambers, or recording the average out-put of atomizers, provides the investigator with prompt notification of anomalies

in the overall operation of equipment as well as an immediate estimate of the extent of sampling necessary to obtain a countable number of bacteria. In so far as limitation of range is concerned, the method has been used to detect single $0{\cdot}3\,\mu$ particles (O'Konski & Doyle, 1955) and desensitization of the photometer circuit should permit measurements in the range of concentration of particles where coagulation becomes the limiting factor.

SUMMARY

The physical behaviour of aerosols atomized into stirred settling chambers, or into rotating drums, was examined by a radioactive tracer method and by a light scatter method. With suitable corrections, there was no difference between the two methods of measuring of physical decay. The tracer method is advantageous in that the data so obtained are on an absolute basis, whereas data from scattered light measurements are relative. The latter method, however, is less time consuming, provides an immediate notification of instrument performance and does not require that material be removed from the aerosol chamber for sampling purposes.

REFERENCES

Bunting, M. I. (1940). A description of some colour variants produced by *Serratia marcescens* strain 274. *J. Bacteriol.* **40**, 57.

Dimmick, R. L., Hatch, M. T. & Ng, J. (1958). Particle sizing method for aerosols and fine powders. *A.M.A. Arch. Ind. Health*, **18**, 23.

Goldberg, L. J., Watkins, H. M. S., Boerke, E. E. & Chatigny, M. A. (1958). The use of a rotating drum for the study of aerosols over extended periods of time. *Amer. J. Hyg.* **68**, 85.

Harper, G. J., Hood, A. M. & Morton, J. D. (1958). Airborne micro-organisms: A technique for studying their survival. *J. Hyg., Camb.*, **56**, 364.

Kethley, T. W., Fincher, E. L. & Cown, W. B. (1957). The effect of sampling method upon the apparent response of airborne bacteria to temperature and relative humidity. *J. infect. Dis.* **100**, 97.

O'Konski, C. T. & Doyle, G. J. (1955). Light scattering studies in aerosols with new counter-photometer. *Anal. Chem.* **27**, 694.