J. Hyg., Camb. (1961), **59**, 487
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Printed in Great Britain

The quantitative assay of mono-dispersed aerosols of bacteria and bacteriophage by electrostatic precipitation

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(*Received* 20 *July* 1961)

INTRODUCTION

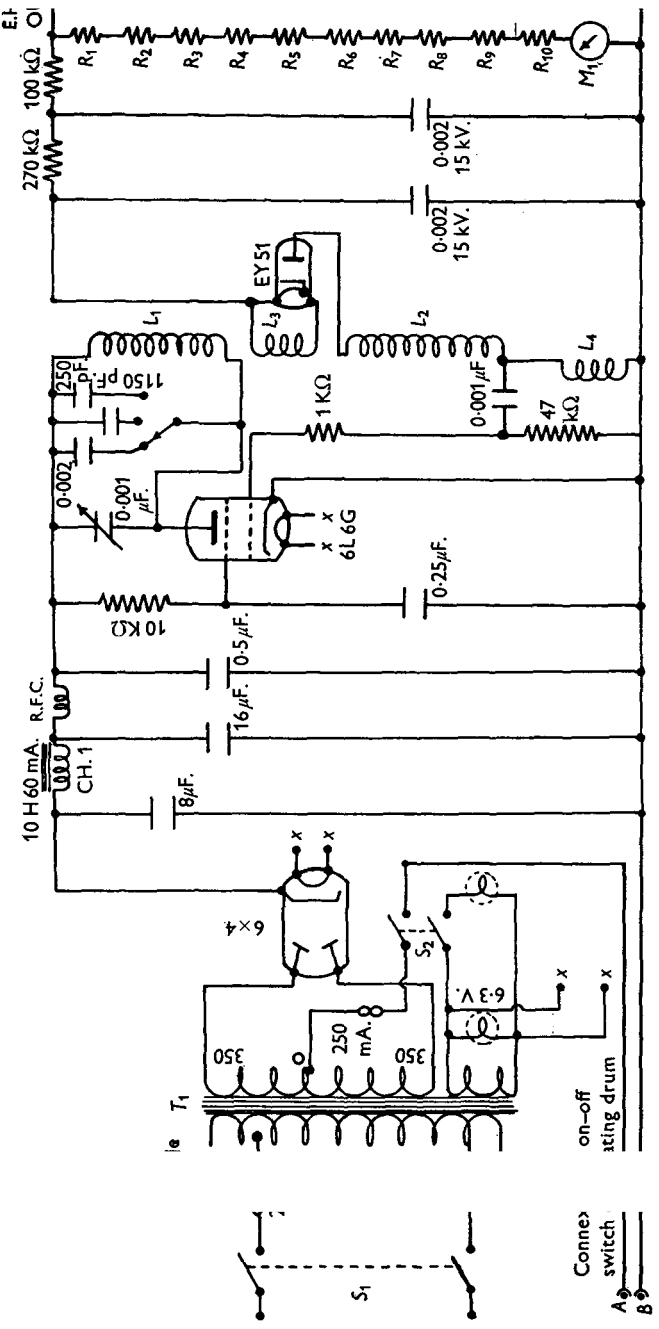
The development of electrostatic methods of sampling for the study of biological aerosols is closely allied to similar work on industrial aerosols. Many modifications of the original invention have been described in the literature reviewed by Green & Lane (1957) and by Batchelor (1960). The type of precipitator used for the estimation of dust and fumes usually consists of a collecting electrode in the form of a tube, and a central ionizing electrode maintained at a high potential. The particles passing through the tube acquire a charge in the electric field and migrate to the outer electrode where they are deposited. To ensure efficient deposition, forced charging of the particles is obtained by a corona discharge between the central electrode and the outer tube. These samplers collect large particles readily but they are also highly efficient in collecting particles less than 1μ in diameter. This latter advantage, combined with their low resistance and high air flow capacity, suggest that electrostatic precipitation methods may fill a requirement for quantitative sampling of bacterial and virus aerosols met by no other method.

Houwink & Rolvink (1957) described a tubular electrostatic precipitator for the assay of bacterial aerosols. Their apparatus consisted of a high-tension electrode mounted centrally inside a cylinder lined with agar or with a fluid. This sampler was not practical for general use, however, since rapid consecutive sampling of aerosols could not be readily achieved, it was not suitable for the assay of small volumes of air, and bacterial colonies on the agar cylinders were difficult to count when the numbers were high, or when opaque media were used. The large volume of collecting fluid used in the liquid sampler also limited the range of aerosol concentrations that could be assessed conveniently. This present report describes three tubular samplers which were constructed in an attempt to overcome these disadvantages and to provide a practical method of electrostatic sampling of bacterial and virus aerosols. Their collection efficiency is compared with that of the slit sampler (Bourdillon, Lidwell & Thomas, 1941) and with both the raised and Porton type impingers (May & Harper, 1957).

CONSTRUCTION

High-tension generator

The high voltage is supplied by a conventional R.F./E.H.T. generator constructed around a coil supplied by Hivolt Ltd. and arranged to have a continuously variable output between 1 and 15 kV. using a 6L6G oscillator valve. The circuit is shown in Text-fig. 1.



Text-fig. 1. uit diagram—E.H.T. generator 2–15 kV. T_1, mains transformer—Gardner type R III/6A. CH 1—Gard type C 231 S_2, switches toggle D.P.S.T. R_1–R_{10}, 10 mΩ 1 % microammeter 0–200 μa. L_1, L_2, L_3, E.H.T. oscillator Hivolt type C 151. L_4, Special R.F. choke supplied with L_{1-3}.

https://doi.org/10.1017/S0022172400039188 Published online by Cambridge University Press

Electrostatic precipitators

The agar collector is shown in Text-fig. 2. It consists of a 2 in. by 12 in. long glass cylinder coated internally with agar, or agar-impregnated paper, and is supported inside a Perspex tube between two screw-caps. One cap carries an air inlet tube and the other a pointed stainless steel electrode, an air outlet and an

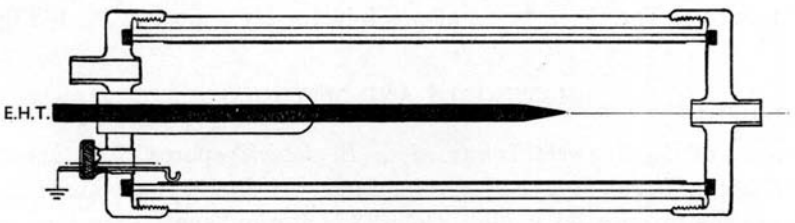


Fig. 2

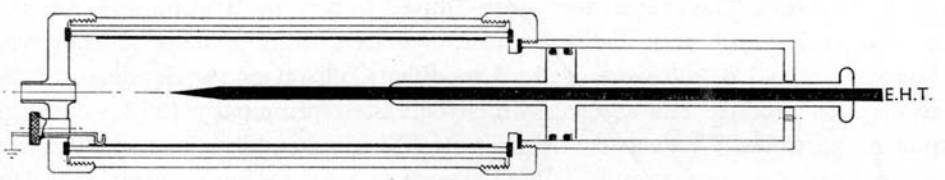


Fig. 3

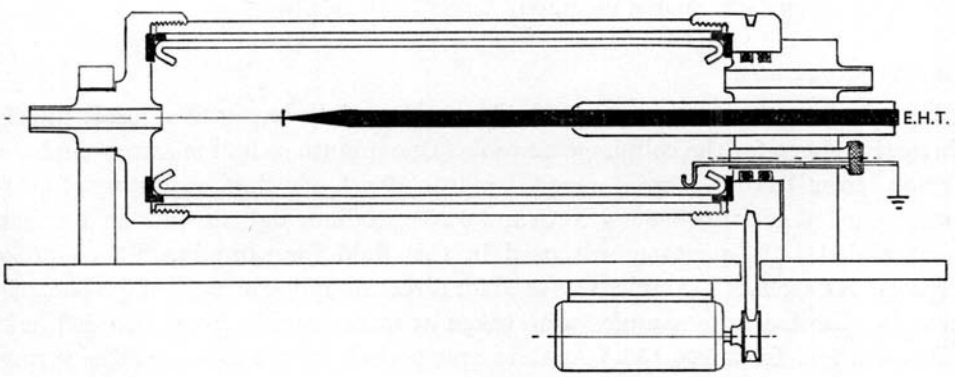


Fig. 4

adjustable earth terminal which is rotated to contact the collecting surface. A positive potential is applied to the electrode, and airborne particles in the controlled air flow through the sampler are deposited on the agar surface. The cap at the air inlet end of the sampler has a coarse thread so that the glass cylinders can be exchanged rapidly, while the high tension, earth and vacuum connections remain attached to the sampler. A modification of this precipitator consists of a syringe attachment for sampling small volumes of air (Text-fig. 3). The electrode is mounted in the piston of the syringe and moves along the length of the collecting surface as an air sample is taken.

The fluid collector is of similar dimensions as the agar model, but it is rotated

horizontally by an electric motor (Text-fig. 4). A glass-collecting cylinder is held inside the Perspex container by rubber centring mouldings in each screw-cap. The cylinders are made with 1 in. intrusions at each end to prevent spillage of the sample fluid. They contain from 5–10 ml. of fluid which is earthed by an adjustable terminal. The high-tension, earth and air-flow connexions are mounted in Perspex pieces which are held stationary in the screw-caps by greased rubber 'O' rings. The electrode for this precipitator is of stainless steel with a polished disk-head $\frac{3}{16}$ in. in diameter. The three types of precipitator are shown in Pl. 1, Fig. 1.

MATERIALS AND METHODS

Micro-organisms

Suspensions of *Bacillus subtilis* var. *niger* (*B. globigii*) spores, *Serratia marcescens* (M 1/48), *Escherichia coli* (162) and *Sarcina lutea* (U 9 25/258) were made in distilled water from growth on solid media. Coli T3 bacteriophage suspensions were prepared from shake cultures of *E. coli* B (Adam, 1950) and by a final filtering through membrane filters. The suspensions were diluted to a concentration not exceeding 1×10^8 organisms/ml. with distilled water, or with a buffer-glycerol solution when *S. marcescens* and *E. coli* were used. A modified Collison spray (Henderson, 1952) was used to produce the aerosols which consisted principally (95 %) of single-organism particles. This was confirmed with T3 coliphage by electron microscope examination of aerosol samples (R. G. Dorman, private communication). Most experiments were done with suspensions of one species of organism but some were made with *B. globigii* spores included.

Sampling apparatus

The raised impinger was used for fluid sampling of the bacterial aerosols and the Porton impinger for the coliphage aerosols. One minute or half minute samples at air flows from 10 to 20 l./min. were taken into 10 ml. of a fluid consisting of 0·1 % peptone, 0·1 % gelatine, 0·85 % NaCl and 0·25 % sodium alginate with an antifoam agent added. 1 % peptone was used in this fluid for sampling T3 coliphage aerosols. A Casella slit sampler was used for direct sampling on agar of the bacterial aerosols. Electrostatic samples were taken at rates ranging from 5 to 50 l./min. and at tensions from 5 to 15 kV. with sample periods from 3 to 60 sec. The syringe precipitator was graduated in 50 ml. volumes, and samples up to 150 ml. were taken at 5 and 8 kV.

The agar tubes for the precipitator were prepared by adding 60 ml. of molten nutrient agar (3 % agar) to the glass cylinders which were then rotated in cold water on a simple spinning device. *E. coli* B was added to the agar during this preparation when the assay of T3 coliphage was required. The surface of the agar was dried at 37° C. for 2 hr. and the cylinders were plugged with gauze and wool for storage. Whatman no. 3 MM hardened paper, in sheets of $6\frac{1}{2}$ in. × 10 in. were used for agar impregnation. The paper was marked in 1 in. squares with lead pencil and rolled inside the cylinders which were autoclaved and then filled with 30 ml. of hot molten agar (1·5 % agar). After saturation of the paper the excess agar was poured off and the cylinders were dried at 37° C.

The sample fluid used in the electrostatic precipitator for liquid collection consisted of 0·2 % Tween 80, 0·1 % peptone, 0·1 % gelatine, 0·85 % NaCl, 0·3 % sodium alginate and 10 % glycerol. This fluid resembled that used in the impinger as closely as possible. It was necessary, however, to include Tween since otherwise a proportion of the sampled particles remained attached to the wall of the glass cylinder. The alginate concentration was then adjusted and glycerol added to provide a uniform coating in the cylinder at various rotation speeds and to reduce the evaporation loss during long-term sampling. Evaporation was of the order of 0·1 ml./10 l. of air sampled at 60 % R.H. and 70° C. Variations made in the fluid volume from 5 to 10 ml., and in speeds from 10 to 30 r.p.m. had no effect on the collection efficiency. The volume of fluid used in the tests reported here was 10 ml. and the rotation speed was 25 r.p.m.

Test system

An aerosol chamber of 14 ft.^{3} capacity was used (Pl. 1, Fig. 1). It contained a small mixing fan, wet- and dry-bulb thermometers and connexions to compressed air and steam. Negative pressure of 1 in. of water was maintained in the chamber with a pump which extracted air through a bacterial filter of merino wool. The relative humidity was adjusted between 60 and 70 % by admitting steam or dry air while the temperature remained between 19° and 21° C. The Collison spray was inserted directly through an orifice in the chamber and the micro-organisms were sprayed for 3 min. Samples of the aerosols were taken with the electrostatic precipitator in parallel with the other sampling apparatus.

The liquid aerosol samples were diluted in buffer solution and plated by a modified Miles & Misra technique on nutrient agar for growth of the bacteria, or on *E. coli* B sandwich plates for T3 coliphage (Adam, 1950). Incubation of the Petri dishes and agar cylinders was for 20 hr. at 37° C. for *B. globigii* and *E. coli*, for 40 hr. at 30° C. for *Sarcina lutea*, for 20 hr. at 30° C. for *Serratia marcescens* and for 6 hr. at 37° C. for T3 coliphage. To prevent water condensing inside the agar cylinders during incubation they were held vertically with the ends sealed with gauze and wool. The agar-impregnated papers were removed from the cylinders after incubation and held on a flat surface for counting purposes.

RESULTS

Agar collection

The collection efficiency of the electrostatic precipitator on agar was determined by sampling a *B. globigii* spore aerosol before and after passage through the sampler. An impinger placed at the exit of the precipitator collected those particles penetrating the sampler. Percentage penetration was expressed in terms of numbers collected at the exit to those sampled from the chamber by an impinger. Precipitator flow rates of 10 and 20 l./min. were used during 5 min. periods at tensions of 5, 8 and 10 kV. It is seen from Table 1 that at 10 kV the collection efficiency was of the order of 100 %.

The effect of electrostatic sampling on the viability of the organisms was determined by comparing the numbers collected with those given by the slit sampler

or impinger. The mean quotients of viable numbers obtained from para samples with the precipitator/slit sampler and with the syringe precipitat impinger are expressed as sampling factors in Tables 2 and 3. More particles w collected by the precipitator than by the slit sampler in all instances (Table

Table 1. *Mean percentage penetration of* Bacillus globigii *spore aerosols in th electrostatic precipitator (agar collection) at various flow rates and tensions*

Flow (1/min.)	Tension (kV.)	No. of tests	Mean % penetration
10	5	9	4·08 (3·06–5·09)
	8	9	0·02
	10	9	0
20	5	9	16·27 (14·18–18·35)
	8	9	3·15 (2·65–3·65)
	10	9	0

Figures in parentheses are 95 % fiducial limits of mean % penetration.

Table 2. *Mean sampling factors obtained from comparisons between the electrostatic precipitator (agar collection) and the slit sampler*

		95 % fiducial limits of mean sampling factor (precipitator/slit)			
		Organisms			
		BG*	SM	EC	SL
		No. of comparisons			
Flow (l./min.)	Tension (kV.)	27	27	18	18
20	10	1·151–1·302	1·136–1·282	1·319–1·438	1·090–1·202
30	12	1·256–1·397	1·125–1·213	1·298–1·387	1·146–1·247
50	14	1·120–1·171	1·090–1·206	1·167–1·233	1·003–1·113
	Means	1·233	1·175	1·307	1·133

* BG, *B. globigii*; SM, *S. marcescens*; EC, *E. coli*; SL, *Sarcina lutea.*

Numbers of coliphage collected with the slit sampler were less than 1 % of electrostatic samples and the slit sampler was therefore not used for ph aerosols. The sampling factors obtained from the syringe precipitator/impin comparisons are shown in Table 3. It is seen that a factor less than 1·0 was obtai with *Sarcina lutea* aerosols. This was due to the agitation of particles occurring the liquid impinger which separated some aggregates of organisms. The viabil of *E. coli* and T3 coliphage was affected by this agitation and greater via numbers of these particles were sampled electrostatically (Table 3).

The distribution of particles on the agar cylinders varied with the shape of electrode, the tension applied to it and with the air sampling rate. A four-pron electrode and a disk-shaped electrode distributed the particles over a sma area than the pointed type finally adopted for this sampler. A flow rate of 10 l./m and tension of 8 kV. distributed particles from an area 1 in. beyond the point of electrode and covered 8 in. of the cylinder length (Pl. 1, Fig. 2). The mov electrode in the syringe sampler produced a similar distribution.

Results with agar-impregnated paper

The efficiency of the electrostatic samplers was not affected when the agar-impregnated paper was used. Bacterial colonies showed no tendency to spread on the paper, but were slightly smaller than on agar after 20 hr. of incubation.

Table 3. *Mean sampling factors obtained from comparisons between the syringe electrostatic precipitator (agar collection) and the impinger*

95 % fiducial limits of mean sampling factor (syringe precipitator/impinger)

		Organisms				
		BG	SM	EC	SL	T3*
Sample volume (ml.)	Tension (kV.)	No. of comparisons				
		27	27	18	18	18
150	8	0·822–1·018	0·920–1·096	1·584–1·702	0·674–0·803	0·945–1·230
100	8	0·960–1·215	0·945–1·246	1·868–2·085	0·840–0·918	1·186–1·332
50	8	1·136–1·309	1·162–1·274	1·974–2·142	0·844–0·985	1·247–1·474
	Means	1·077	1·107	1·892	0·844	1·236

* Tests with T3 coliphage were made at 5 kV tension and at 80 % R.H.

Table 4. *Mean percentage penetration of* Bacillus globigii *spore aerosol in the electrostatic precipitator (fluid collection) at various flow rates and tensions*

Flow (l./min.)	Tension (kV.)	No. of tests	Mean % penetration
10	8	9	5·36 (2·94–7·77)
	10	9	0·02
	12	9	0
20	10	9	2·44 (1·79–3·09)
	12	9	0·71 (0·63–0·79)
	13	9	0·27 (0·16–0·38)

Figures in parentheses are 95 % fiducial limits of mean % penetration.

Numbers of colonies up to 5000 per air sample were readily counted. An example of the growth of *S. marcescens* on paper is shown in Pl. 1, Fig. 2. With non-pigmented organisms, the colour contrast on paper was improved by the addition of a fermentable carbohydrate and an indicator (aniline blue) to the agar used for impregnation. Blood cells could also be added to the agar but zones of haemolysis were difficult to see except when the organisms were markedly haemolytic. The papers could be preserved as a permanent record by treatment with formalin vapour followed by a drying period during which the bacterial colonies were adsorbed by the paper and could not then be removed.

Fluid collection

The collection efficiency of the fluid sampler was determined as for the agar model, but a higher tension was necessary as seen in Table 4. Mean sampling factors obtained from comparisons between the precipitator and impinger are shown in

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Table 5. It can be seen that the packets of *Sarcina lutea* were broken up to the same extent in the fluid precipitator as in the impinger. The effect of agitation in the impinger again lowered the viable recovery of *E. coli* and T3 coliphage aerosols and thus increased the sampling factors obtained.

Table 5. *Mean sampling factors obtained from comparisons between the electrostatic precipitator (fluid collection) and the impinger*

		95 % fiducial limits of mean sampling factor (precipitator/impinger)				
		Organisms				
		BG	SM	EC	SL	T3*
		No. of comparisons				
Flow (l./min.)	Tension (kV.)	45	45	30	30	30
5	8	1·132–1·256	1·156–1·276	1·598–2·081	1·036–1·254	1·442–1·61
10	10	1·134–1·212	1·065–1·173	1·696–2·052	1·097–1·393	1·291–1·70
20	12	1·188–1·249	1·056–1·224	1·589–2·066	1·062–1·255	1·349–1·75
30	14	1·152–1·314	1·121–1·250	1·336–1·635	1·056–1·252	1·238–1·56
40	15	1·069–1·228	1·025–1·126	1·347–1·600	1·035–1·228	1·518–1·73
	Means	1·193	1·147	1·700	1·167	1·521

* Tests with T3 coliphage were made at 80 % R.H.

Table 6. *The electrostatic precipitator/impinger sampling factors obtained at various humidities from aerosols of* E. coli *and T3 coliphage, each containing* Bacillus globigii *spores*

		Sampling factors (precipitator/impinger)			
R.H. (%)	Temp. (°C.)	*E. coli*	Spores	T3 phage	Spores
60	70	1·877	1·179	183·000	1·106
80	70	1·334	1·136	1·814	1·050
90	70	1·311	1·158	0·870	1·079

The effect of humidity on the sampling factor

During preliminary experiments with T3 coliphage it was found that the aerosol humidity markedly influenced the viable recovery of phage from impinger samples. A similar effect was noted with *E. coli* aerosols but it was less marked. Suspensions of *E. coli* and T3 coliphage, each containing *B. globigii* spores as a tracer organism, were subsequently sprayed at three levels of relative humidity. Parallel samples of the aerosols were taken with the fluid precipitator and with the appropriate impinger; the raised type for *E. coli* and the Porton impinger for the phage. The sampling factors obtained suggest that the impinger viability of *E. coli* and T3 coliphage increased with the R.H. (Table 6). The collection efficiency of the samplers, as shown by the spore results, was not affected by changes in humidity. At 90 % R.H. the impinger recovery of coliphage was greater than that from the fluid precipitator samples. This latter phenomenon was attributed to the lethal action of ozone produced in the electrostatic sampler.

The effect of ozone in the fluid electrostatic precipitator

It is known that bacteriophage are extremely sensitive to ozone (Adam, 1959) and that this gas requires a high humidity for its action (Elford & Van den Ende, 1942). It seemed likely therefore that ozone was responsible for the lowered recovery of phage at humidities greater than 80% R.H. The amount of ozone produced in the precipitator depended on the tension applied to the electrode. At 90% R.H. a reduction from 10 to 7 kV. improved the precipitator/impinger sampling factor from 0·87 to 0·97. This measure had little effect on sampling efficiency since the penetration of phage through the precipitator was found to be approximately half that obtained with *B. globigii* spores. Ozone production was also related to the type of discharge emitted by the electrode. A pointed electrode emitted a spiked discharge which produced lower recoveries of phage than the corona given by the disk-head type finally adopted. The coliphage particles were protected from ozone when they were sprayed from suspensions containing 0·1% peptone. A maximum tension of 15 kV. could then be applied without effect on their viability. Since the peptone in the collecting fluid had no such protective action, death of the particles occurred only whilst airborne in the sampler. Ventilation of the collecting tube occurred at a rate depending on the air flow when sampling commenced. It is likely therefore that the ozone concentration was at an active level only during the first few seconds of the sample period.

DISCUSSION

The theoretical advantages of electrostatic precipitation methods are that samples of either mono-dispersed or aggregated bacteria or viruses can be taken on to solid or liquid media without trauma; minimal limitation is imposed by aerosol concentration and sample size; and collection efficiency is very high. The apparatus described in this report fulfills these requirements and appears to have largely eliminated the shortcomings of that described by Houwink & Rolvink. The disadvantages appear to be those inherent in any high voltage equipment and in the necessity for some technical skill in preparation and operation. Its potentialities in all fields of microbiological air sampling however, would appear to compensate for these disadvantages.

SUMMARY

Three tubular electrostatic precipitators are described for the assay of bacterial and bacteriophage aerosols. Particles can be sampled directly on agar or on agar-impregnated paper in a wide range of aerosol concentrations, or sampled into a fluid for the assay of airborne micro-organisms. Rapid consecutive sampling is possible with the samplers and a collection efficiency approaching 100% is readily obtained. Their sampling efficiency is compared with that of the slit sampler and the liquid impinger.

We are indebted to Mr H. A. Druett who suggested the use of a syringe sampler, to Mr S. Peto for statistical treatment of the data, and to Mr C. M. R. Pitman for excellent technical assistance. The glass collecting tubes for the precipitators were made by Mr W. I. P. Nelson.

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EXPLANATION OF PLATE

Fig. 1. The three types of electrostatic precipitator in the foreground. The precipitator for fluid collection is shown connected to the high-tension generator which is on top of the aerosol chamber. The spinning device used for preparation of the agar cylinders is shown on the right of the generator.

Fig. 2. An electrostatic sample of *S. marcescens* aerosol on agar, and a similar sample grown on agar-impregnated paper.

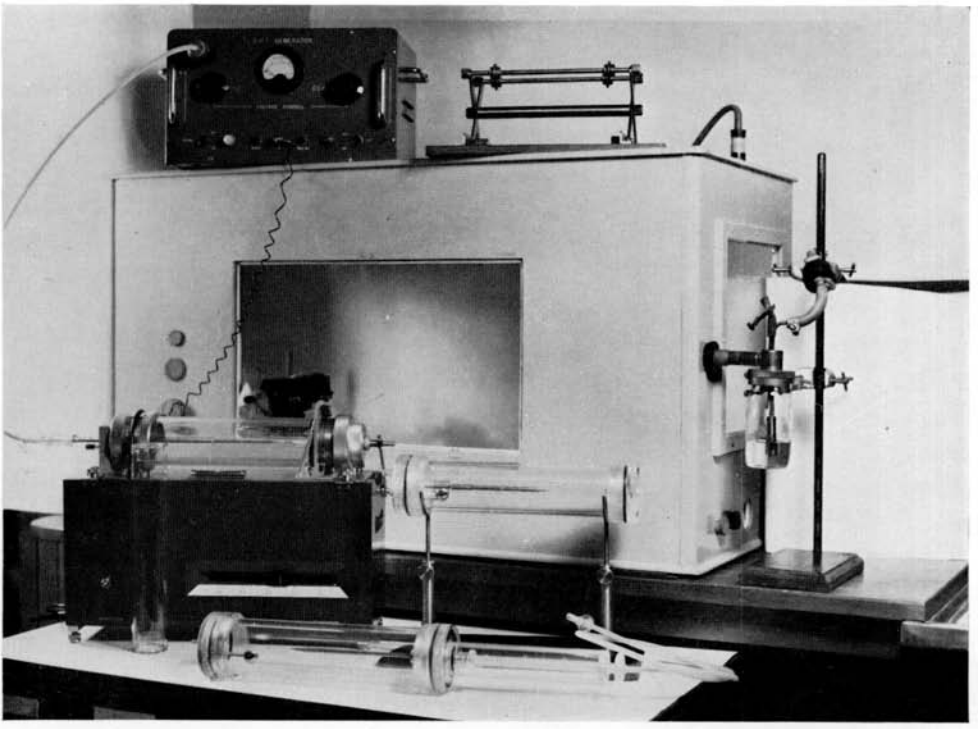

Fig. 1

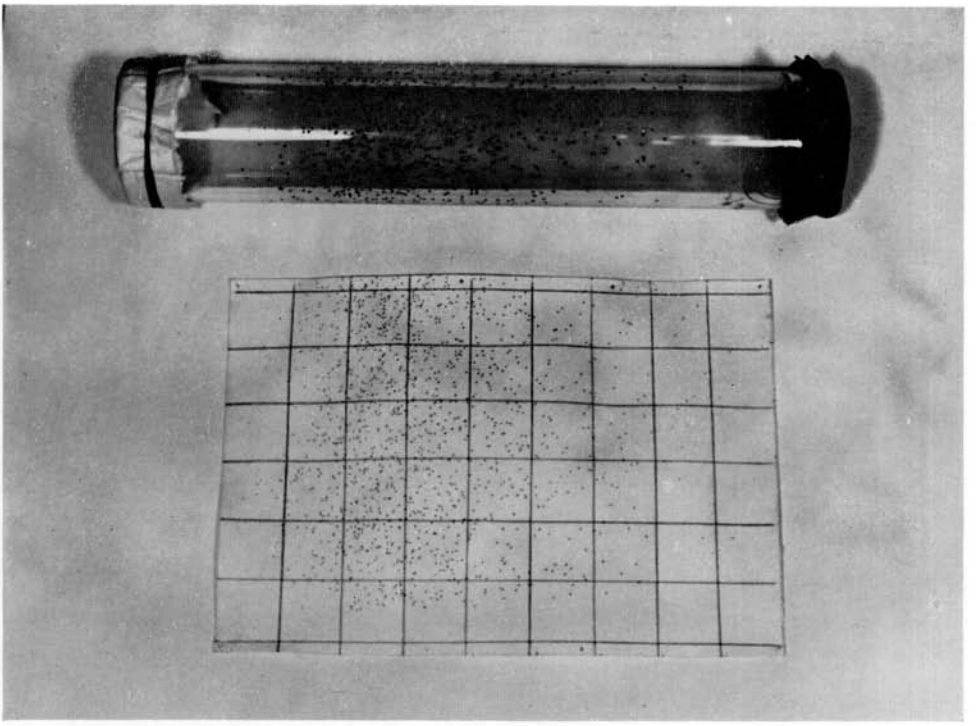

Fig. 2

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