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ON THE ISOLATION OF AIR GERMS USING FINE-PORE FILTERS

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The cytology of microorganisms floating freely in atmospheric and extra-atmospheric space has so far been subject to relatively little systematic investigation. For instance, we know very little about the structures and mechanisms that enable a whole series of microorganism types to withstand long-lasting periods in the atmosphere without any loss of vitality, while other microorganisms, under the same conditions, mostly lose their capability to multiply after a very short time.

To prepare the way for investigations in this field, we tried to determine in this work just exactly to what extent filtration methods are suitable for the isolation of air germs and what must be taken into consideration in the use of such methods.

Filtration methods, within the framework of microbiological aerosol analysis methodology, have by far not been used in as manifold a fashion as in dust measurement technique; impinger methods proved to be superior in most cases to the air germ filtration methods tested so far.

Kruse (1948) isolated air germs with the help of membrane filters which he directly placed on solid nutrient media, following the filtration process, without any further treatment, and which he then incubated until the development of macroscopically recognizable colonies. This kind of method, at best, made it possible to determine the number of germ-laden particles contained in the air volume examined. Total germ counts however cannot be performed in this fashion (see Albrecht, 1957). By the way, the method of Kruse can be used in establishing only relatively small germ counts, in each case, as is true of all of the other direct methods. Excessively high germ counts give us colony overlaps [colony overlaps result in excessively germ counts] and, under certain circumstances, we also get antagonistic effects; these excessively high germ counts, moreover, can

produces considerable subjective counting errors (Niemela, 1965). But it is precisely in aerosol investigations that we must have very voluminous numerical data in order to get statistically reliable experimental results. In order to convert the Kruse method into an indirect method, Humphrey and Gaden (1955) reduced the filters employed for filtration by mechanical means; Michals and Spurny (1959) dissolved them with methylcellosolve. An attempt was also made to use water-soluble filters for air germ filtration (see Wladawez, 1957; Mitchell et al, 1954; Nolte and Spendlove, 1956).

The Gottingen membrane filter company has been producing soluble gelatin filters for a number of years. Because these filters are easily handled and do not require any great effort, it appeared a good idea to investigate their applicability thoroughly.

Material and Methods

Air Germ Filtration. We used gelatin and membrane filters with a diameter of 50 mm in open air filtration units made by the Gottingen Membrane Filter Company. The membrane filter was sterilised by boiling 3 times and was then dried at 50° C. To measure the air through-put, we used measurement tubes supplied by Fischer and Porter, Gottingen.

Dissolution of Gelatin Filter. The gelatin filters were dissolved, in each case, in 50 ml of 0.4% Na_2HPO_4 solution, at 38°-40° C. The resultant suspensions were stirred for about 10 minutes by means of sterile magnetic stirring rods and they were then wholly or partly filtered through sterile membrane filter MF 30 with grill network [grid]. The membrane filters were then incubated on peptone-glucose-agar (see below).

Impinger Method. We used midget impingers (air through-put: 3.78 l/min), AGI impinger (interval between nozzle opening and container [vessel] bottom = 30 mm: "AGI-30"; air throughput: 12 l/min) and modified Bronn impinger impingers (see Windisch, 1965; inside diameter of inlet pipe about 7 mm, nozzle diameter about 1 mm; air throughput: 12 l/min). The impinger solution (10 ml per midget impinger, 30 ml per AGI unit, 65 ml per Bronn impinger) consisted of a watery solution of 0.2% gelatin and 0.4% Na_2HPO_4, for use in midget and AGI units, mostly with the addition of 0.02% silicon defoamer Bayer E and 0.01% Antifoam AF (Dow Corning). The midget impingers were used in combination with the sequential sampler of the Gelman Instrument Company, Chelsea, Michigan; Aeromat membrane pumps were used to operate the 2 other impinger types. After completion of the collection phase, the entire impinger solution or a certain portion thereof was in each case filtered through a sterile membrane filter MF 30 with grid net, which was then incubated on peptone-glucose-agar.

Nutrient Medium. Peptone 0.5%; glucose 0.5%; NaCl 0.3%; $FeSO_4 \cdot 7H_2O$ traces; in case of solid nutrient media: agar 1.5%, pH 7.2-7.4.

Aerosol Channel [Canal, Duct]. We used an aerosol duct built according to verbal information from Goetz, Pasadena. This channel [duct] was 7.35 m long in our experiments (without atomizer mouth). It consisted of a glass pipe with an outside diameter of about 60 mm and a wall thickness of about 1.5 mm. We atomized with metal-nozzle atomizers provided the Meyer Company of Bad Ems, with air throughput of mostly 6 l/min. Right after the atomizer, an additional 6 liters of air per minute were added to the duct as supplementary air, through a device designed by Goetz. This additional air contributed to the development of an almost laminar flow. The microorganism cells thus each time spent about 1 minute 27 seconds in the aerosol duct. To dose the air volume flowing into the duct, we used measurement pipes by the Fischer and Porter Company with needle valve. The volume of suspension atomized per experiment was determined mostly by means of weight difference determination. During the last experiments, we used an atomizer which was provided with a continual feed system and a calibrated measurement pipe [tube]. The supplementary air, which in some of the experiments had been pre-filtered through MF 30, came from compressed-air bottles [tanks]. The establishment of isokinetic flow conditions did not appear to be absolutely required in our investigations. Surplus aerosol could leave the duct practically without any stagnation.

Test Organisms. The test organisms used in the experiments with the aerosol duct were Serratia marcescens, Strain 1534, from the Institute of Microbiology of the University of Gottingen, as well as Bacillus subtilis var niger (Bacillus Globigii), ATCC Strain 9372, after at least 8 days of prior cultivation in peptone-glucose nutrient solution (100 ml in 300 ml-Erlenmeyer flasks) ([1] Bacteria cells from growing cultures tolerate atomization extremely poorly; see Goodlow and Leonard, 1961). To kill the vegetative cells, the cell suspensions of Bac subtilis var niger were dipped in boiling water for 30 seconds. The concentration of cells capable of multiplying was determined with the help of dilution series, filtration through MF 30 with grid network and incubation on peptone-glucose-agar, by counting the macroscopically recognizable colonies. As Uehleke (1953; see also Uehleke and Poetschke, 1957) were able to determine, this method is clearly superior to the plate-casting [plate-pouring] method.

Evaluation of Experiment. The experiment was evaluated partly by counting the colonies that became macroscopically visible and partly as a result of microscopic investigation of stained membrane filters that had been rendered transparent.

To facilitate the macroscopic count in preparations with predominantly colorless microorganism colonies, contrast staining with 0.01% watery malachite-green solution was performed in accordance with the data in the Millipore Application Data Manual (ADM-40; 1961).

For the microscopic contrasting of the preparations, we tried methylene blue staining (Jannasch, 1953, 1958), methylene blue-fuchsin staining (Millipore Application Data Manual ADM-40), methylene blue-carbolerythrosin staining (Sujkowa, 1959), carbolerythrosin staining (Niemela, 1965) as well as Gram staining (Weinfurtner and assoc, 1956).

The transparency of the membrane filters necessary for microscopic investigations was achieved either by soaking with immersion oil or by the dissolution of the pore structure with methylcellosolve, respectively, with absolute alcohol and ether vapor (Rost, 1965).

When the latter two methods are used, the outlines of the aerosol droplets, absorbed on the filters, are caused to disappear, along with the pore structure. These droplets can therefore be observed only when we use the immersion oil method.

Results

1. Characterization of Gelatin Filters Provided by the Membrane Filter Company

The first gelatin filter lots, procured from the Membrane Filter Co, revealed little uniformity with respect to their properties. In connection with the absorption of particles atomized from a 2% Congo Red suspension it was found that only about half of all the filters examined were covered on the front side with a homogeneous red-brown dyestuff layer and that they simultaneously retained an unstained reverse side. The others either were highly resistant against the air current and revealed bright strips after filtration, indicating that there had been no filtration effect at all here, or they had pores of such size that their reverse side likewise was definitely stained. These gelatin filters were mostly considerably thicker than the membrane filters (the latter are only about 150 millimicrons thick) but they revealed considerable thicknesses also among themselves. Measurements on 10 filters gave us thicknesses between 210-425 millimicrons in the border zone and 260-435 millimicrons in the middle region.

Coulter Counter measurements revealed that the nonhomogeneity of the filters was due not only to their thickness but also to the nonhomogeneity of the gelatins used.

On the basis of these findings, the gelatin filter production method was improved so that we now have gelatin filters of quite uniform filtration effectiveness available. These filters are not as thick and the thickness fluctuations likewise are smaller. Measurements conducted with 8 filters from a lot [delivered] in October 1965 gave us thicknesses between 200-280 millimicrons in the border zone and 190-290 millimicrons in the middle region. Thinner filters (140-155 millimicrons) likewise proved useful in laboratory experiments. With the help of the Royco Particle Counter and through the evaluation of electron-microscope pictures it was discovered that all gelatin filters suitable for air germ filtration have an efficiency of almost 100% for Latex and Congo Red aerosol particles with a diameter of 0.5 millimicrons.

Experiments intended to determine the efficiency of the gelatin filter with respect to bacteria aerosols were conducted with the help of Bacillus subtilis var niger in the aerosol duct. Here 2 filters were superposed and placed in the air filtration equipment used for exhaust purposes at the duct

exit: in front we had the filter whose permeability was to be tested and after that we had one gelatin filter. Overlooking the number of bacteria cells passing through the second filter, we obtained the following permeability percentage for the filter tested:

$$100 - E = \frac{K_2 \cdot 100}{K_1 + K_2} (\%)$$

respectively, an efficiency percentage of

$$E = 100 - \frac{K_2 \cdot 100}{K_1 + K_2} (\%).$$

whereby
K_1 = number of cells capable of reproducing on the forward filter and
K_2 = number of cells capable of reproducing on the rear filter.

In the computation of the permeability of the MF 500 and MF 100 membrane filters tested, we took the values for K_1 which were determined in connection with the testing of the gelatin filters.

The dependence of the E-values on the filtration speed in the range between 2 and 12 cm · sec^{-1} is shown in Figure 1. As we can see, the efficiency of the gelatin filters examined is between that of the MF 500 and MF 100 membrane filters. This is in keeping with the results of the above-mentioned investigations with Congo Red and latex aerosols.

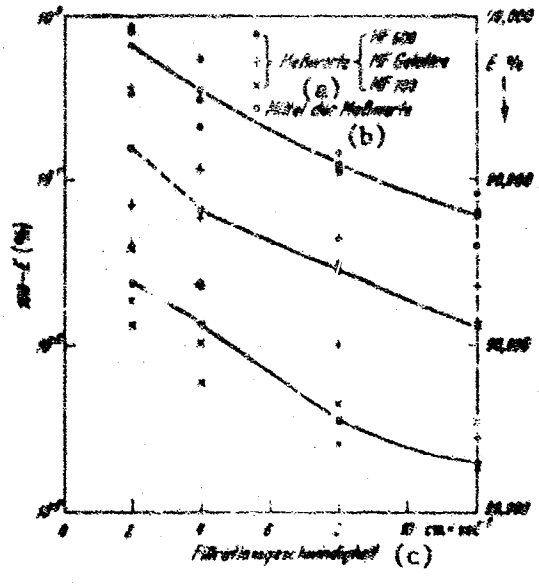

Figure 1. Efficiency of MF 500, MF gelatin and MF 100 for atomization from aerosols of Bac subtilis, var niger, prepared from 0.85% NaCl solution, computed for filtration speeds of 1.99-11.95 cm · sec^{-1}.
Key: a. Measurement values
b. Average of measurement values
c. Filtration speed

Unfortunately, ethylene oxide sterilization causes a gradual reduction in the filter solubility. Fresh gelatin filters or gelatin filters that were only a few months old, on the other hand, can be used under almost all conditions, for instance, also in case of minus temperatures; only aerosols with a very high moisture content can impair the efficiency of the filters by softening and enlarging the pores.

([2] The speed of perforation and the size of the holes resulting can be determined very easily by taking a membrane filter which has been placed under the gelatin filter during aerosol filtration and then staining it according to Sujkowa (1959) and examining it under the microscope after soaking in immersion oil.)

2. Air Germ Yield Resulting from the Use of Gelatin Filter Methods

Figure 2 shows us the disadvantages of the Kruse membrane filter method: in macro-colony counts, we can never determine the total number of the microorganism cells that reached the filter because in many cases only one single macro-colony becomes recognizable in places where several microorganism cells have hit the filter. The error due to such overlap effects grows as the density of the microorganism cells absorbed on the membrane filter increases; indeed, it grows very rapidly, up to a point at which macro-colony counts become meaningless. The use of the abovementioned gelatin filter method however does not involve these difficulties because in this case it is possible to produce any desirable dilutions and to reduce the larger particle aggregates quite extensively.

Compared to the drying effect, connected with the filtration process, the normal components of the natural air microflora are so resistant that their vitality is not impaired to any great extent as a result of this. ([3] The situation however is different with respect to the significance of the drying effect in the methods of impaction on solid nutrient media surfaces. Here we get mostly relatively high flow speeds and there is a possibility that the structure of the nutrient medium might be altered unfavorably due to the drying-out process.) In the course of comparative general experiments in the vicinity of the Institute of Aerobiology it was therefore possible, with the help of the gelatin filter method, to establish in most cases more germs per unit of air volume than when we use an AGI-30 impinger. The ratio between the number of growing bacteria and yeast colonies and the number of mold fungus and actinomycete colonies differed hardly from the ratio determined in parallel experiments with Koch's plate method.

([4] A detailed publication on this subject is scheduled for a later date.)

Investigations conducted on board boats in the Gulf of Naples (elevation about 1 m above sea level) on the other hand revealed considerably smaller percentages of bacteria and yeast colonies in the gelatin filter method, than in the plate method. Obviously, many sensitive maritime bacteria had fallen victim to the drying action. ([5] These investigations [results so far unpublished] were conducted with the support of the German Research Association.)

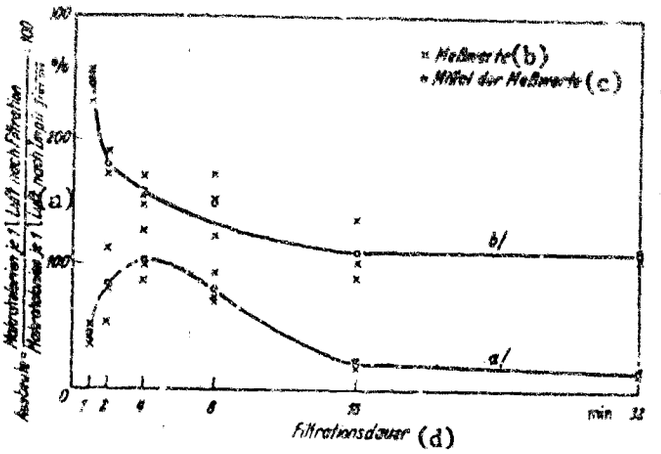

Figure 2. Air germ yield resulting from use of Kruse membrane filter method (a, MF 30 with grid, boiled in distilled water for 3 · 10 min, and dried in sterile Petri dishes) and from gelatin filter method (b) in the rabbit hutch of the Institute of Aerobiology (midget-impinger values = 100%, mostly between 15 and 80 colonies per liter of air). In each case we filtered 12 l of air per minute. The germ content fluctuations were generally considerably smaller in the air of the rabbit hutch than on the outside but the hutch air appeared to contain relatively many germs that were sensitive to longer filtration. This might partly explain the rather considerable initial drop in the curve at b. The rather strong initial rise in curve a seems to be due to the properties of the membrane filter. When the 1- and 2-minute filtration experiments were repeated several times, we obtained averages which did not essentially differ from those given here; the distribution [spread] of the individual measurement data however was quite considerable in most cases. Greater measurement accuracy could be achieved only in case of filtration experiments lasting at least 4 minutes.

Key: a. $\text{Yield} = \frac{\text{macrocolonies per l of air after filtration}}{\text{macrocolonies per l of air after impinging}} \times 100$

b. measurement values
c. average of measurement values
d. duration of filtration

The normal components of the air microflora can withstand many days of adherence to gelatin filters without a significant vitality loss (see Figure 3). It is therefore possible to allow a relatively long interval of time to pass between the collection phase and the laboratory investigation as such.

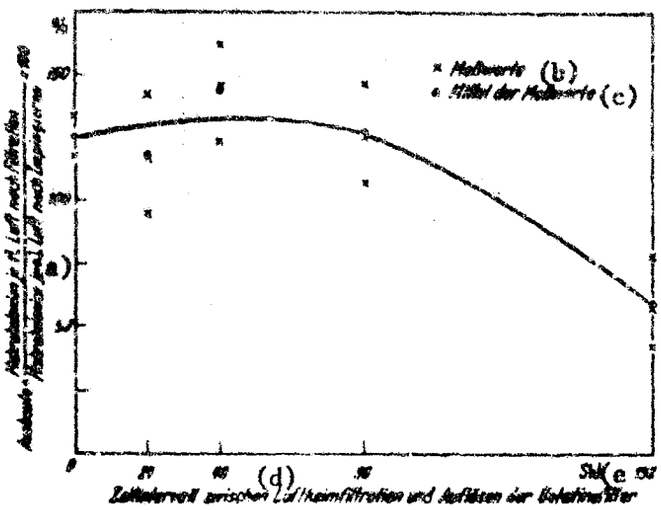

Figure 3. Colony yield as a function of the duration of time interval between air germ filtration and dissolution of the filters. The air germ filtration was performed in the rabbit hutch of the Aerobiology Institute (midget-impinger values: 46.9-113.0 colonies per 1 of air). Duration of collection phase: 5 minutes. Collection output: 12 l of air/minute. The gelatin filters were placed in sterile Petri dishes, after the filtration phase, at about 20° C.

Key: a.

$$\text{Yield} = \frac{\text{macrocolonies per 1 of air after filtration}}{\text{macrocolonies per 1 of air after impinging}} \times 100$$

b. Measurement values
c. Average of measurement values
d. Time interval between air germ filtration and dissolution of gelatin filters
e. Hours

In similar investigations, the impacting effect proved to be hardly relevant at filtration speeds of 60 cm sec^{-1} (see Figure 4).

([6] The initial rise in the curve is mostly explained by the fact that the aerosol filtration equipment in these experiments was installed with the opening facing downward so that the suction on the filter worked against the ground [terrestrial] acceleration.)

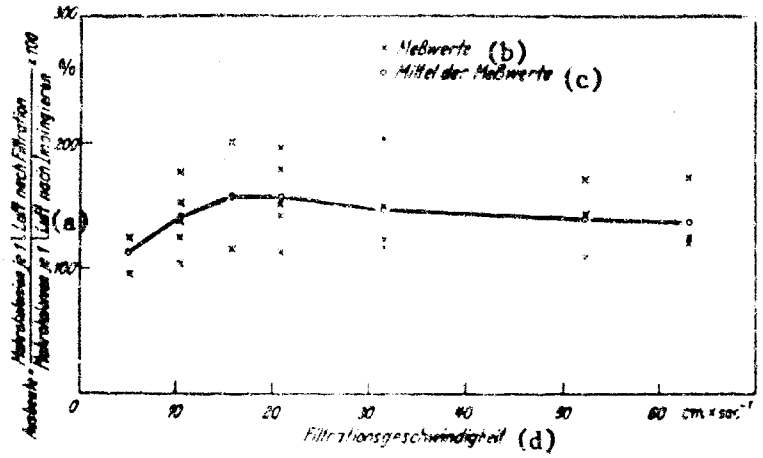

Figure 4. Colony yield as a function of the filtration speed. Air germ filtration performed in rabbit hutch of Aerobiology Institute (midget-impinger values: 15.4-69.2 colonies per liter of air), collection output — 5, 10, 15, 20, 30, 50, and 60 liters of air. A total of 300 l of air were filtered per experiment.
Key: [a, b, c, same as Figures 2 and 3]
d. Filtration speed

Microorganisms, which lose their reproduction capability after a relatively short stay in the atmosphere, are also sensitive with respect to the filtration process. Table 1 shows us the results of a typical aerosol experiment for the comparative determination of the yields of reproducible cells of Serratia marcescens when we use the filtration and the impinger methods. A number of similar experiments revealed that the yield in terms of Serratia cells capable of reproducing — a yield to be obtained through filtration experiments — increased as the water content of the aerosol increased and that it dropped as the atomized water volume decreased. The ratio between the filter yield and the impinger yield in connection with the atomization of cells from growing cultures was reduced in size by whole powers of ten.

Considerably different findings resulted from experiments in which spores of Bac subtilis var niger were atomized (see Table 2). Here, filtration produced larger colony yields than impinging. On the basis of the fact that the aerosol contained a larger number of aggregations consisting of several cells, Table 2 in particular illustrates the advantage of using water-soluble filters which facilitates the comminution of a considerable part of the cell aggregations. When surface-active substances are used, the colony yield in such cases will presumably increase even further (see Jones and Jarnasch, 1959). The error margins given in Tables 1 and 2 can probably be cut back considerably through improvements in the experimental methodology, particularly in experiments with such sensitive bacteria as Serratia marcescens.

TABLE 1

YIELD OF REPRODUCING CELLS OF SERRATIA MARCESCENS IN CASE OF ISOLATION WITH THE HELP OF FILTRATION AND IMPINGER METHODS (AEROSOL DUCT EXPERIMENTS)

Sammelverfahren (a)	Ausbeute (%) (b) Vers.-Nr. (c)								M	σ	$\frac{\sigma \cdot 100}{M}$
	1	2	3	4	5	6	7	8			
Filtration durch MF 30 mit Gitternetz (d)	1,15	1,13	1,01	0,98	1,07	0,90	0,99	1,04	1,03	0,03	2,83
Filtration durch Gelatinefilter (e)	1,01	1,03	0,96	0,93	1,01	1,08	1,07	0,97	1,01	0,02	1,84
Impingerverfahren: AGI-30 (f)	13,58	11,18	10,75	11,56	12,04	11,15	12,42	10,07	11,47	0,41	3,54
Impingerverfahren: Impinger nach Bronn (g)	14,25	13,17	15,06	13,89	14,07	13,58	14,36	13,86	14,01	0,20	1,45

Key: a. Collection method
b. Yield (%)
c. Experiment number
d. Filtration through MF 30 with grid
e. Filtration through gelatin filter
f. Impinger method: AGI-30
g. Impinger method: Impinger according to Bronn

$$\text{Yield} = \frac{\text{Number of isolated cells capable of multiplying, per 1 of air} \times 100}{\text{Number of atomized cells capable of multiplying per 1 of air}}$$

M = average value

= average error in mean values; = average error percentage

v = absence of individual measurements with respect to M; n = number of individual measurements

[See page 11 following, Experimental Conditions]

[Continuation of Table 1, page 10]

Experimental conditions. Atomized suspension: 45,000 cells capable of reproducing in 0.188 ml of 0.85% NaCl solution per minute. Air supplied, total: 15 l/min. Air volume suctioned in through collecting equipment: 12 l/min, each time. Duration of experiment: 2 min, each time. Temperature: 20° C.

Of the suspensions resulting from the dissolution of the gelatin filters, 50%, in each case and of the impinger solutions, 10%, in each case, were used for experiment evaluation. The number of colonies to be counted on the following day, per preparation, was between 335 and 1,084. As a result of the early count (malachite-green method) there were no inaccuracies due to overlap effects.

The all-glass impinger, designated as AGI-30, was the commercially available equipment unit produced by the Millipore Filter Corp. The relatively large scatter of measurement values obtained here was mainly due to the fact that the impinger solution did not contain any antifoam addition.

The impinger according to Bronn essentially was based on data of Windisch (1965). The inside diameter of its intake [inlet] pipe was barely 7 mm and the nozzle diameter was 1 mm. In this unit, the aerosol particles are impinged in a tangential direction, similar to the Shipe impinger (see Shipe, Tyler and Chapman, 1959; Tyler, Shipe and Painter, 1959), whereby the solution is given a rotating motion.

TABLE 2

YIELD IN REPRODUCIBLE SPORES OF BAC SUBTILIS VAR NIGER IN CONNECTION WITH ISOLATION WITH THE HELPO OF FILTRATION AND IMPINGER METHODS (ARNOLD DUCT EXPERIMENT)

Sammelverfahren (a)	Ausbeute (%) Vers.-Nr. (b) (c)										
	1	2	3	4	5	6	7	8	$\bar{x}$	σ	$\frac{\sigma \cdot 100}{\bar{x}}$
Filtration durch MF 20 mit Gitternetz (d)	24,90	25,20	25,00	25,10	24,80	26,30	25,80	24,70	25,10	0,19	0,49
Filtration durch Gelatinefilter (e)	28,00	28,30	28,20	27,60	28,10	27,70	27,80	28,20	28,10	0,19	0,77
Impingerverfahren: AGI-30 (f)	24,35	24,10	24,30	23,70	24,75	24,90	25,25	24,45	24,45	0,16	0,65
Impingerverfahren: Impinger nach Brown (g)	24,75	25,40	24,85	24,90	24,00	23,85	24,70	24,85	24,70	0,15	0,62

Key: a. Collection method; b. Yield (%); c. Experiment number; d. Filtration through MF 30 with grid; e. Filtration through gelatin filter; f. Impinger method: AGI-30; g. Impinger method: impinger according to Brown

Experimental conditions. Atomized suspension: 2,500 spores capable of reproducing in 0.250 ml of 0.85% NaCl solution per minute. Total air volume introduced: 15 l/min. Air volume suctioned in through collection equipment: 12 l/min, in each case. Duration of experiment: 2 minutes, in each case. Temperature: 20° C.

Of the suspensions resulting from the solution [dissolution] of the gelatin filters, 50% in each case were used for evaluation and the impinger solutions were filtered completely through the membrane filters earmarked for incubation. The number of colonies to be counted on the following day, per preparation, was between 275 and 516.

In this experiment, the antifoam AF (Dow Corning) impinger solutions contained a concentration of 0.1 ml/l.

After atomisation, cells of Serratia marcescens lose their reproduction capacity relatively quickly not only during their stay in the atmosphere but also during the filtration process; they tolerate preservation in gelatin filters quite poorly. Since their rapid mortality is essentially due to the drying action, we can increase their survival capability by adding substances which more or less slow down the process of cell drying or which preserve the cells against death due to drying in some other fashion.

([7] Webb (1960) assumed that meso-Inosit and other substances, on the basis of their steric configuration, are in a position to replace the water molecules within the cells and thus to protect the albumin structures. On the other hand, however, Zimmermann (1962) found that atomised Serratia cells are protected considerably better by those sugar molecules which do not pass through the cytoplasm membrane than by means of sugar molecules that have good permeation properties.)

Figure 5 illustrates the influence of various substances atomized along with the bacteria upon the survival capability of the cells, after absorption on gelatin filters. Cells atomized from distilled water died off extraordinarily rapidly here and atomisation from 0.85% NaCl solution produced a mortality curve that is typical for monomolecular reactions with a kill rate of about 3.65%/minute. ([8] In mortality curves with the form

$C_t = C_o \cdot e^{-kt}$ the kill rate per minute is $= 100\left(1 - e^{\frac{\ln C_t - \ln C_o}{t}}\right)$, whereby

C_o = germ concentration at the start of the curve and
C_t = germ concentration after time t (min) (see Beebe, 1959).)

On the other hand, additions of powdered skim milk, meso-Inosit, and silicon oil produced a considerable lengation of the lifetime of some of the isolated bacteria.

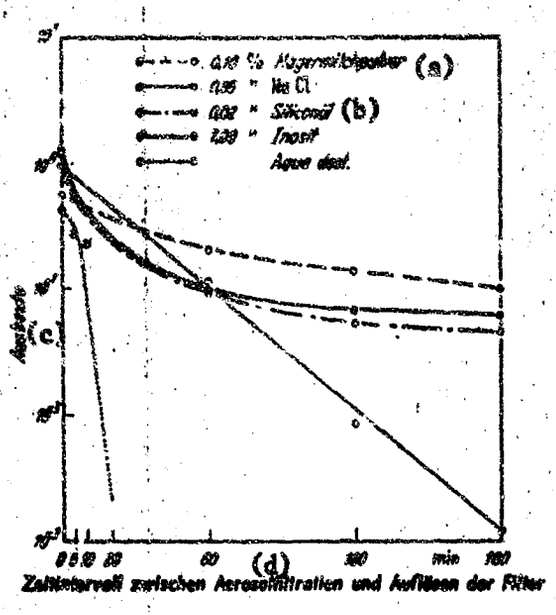

Figure 5. Colony yield as a function of the duration of the time intervals between air germ filtration and dissolution of the filters. Aerosol duct experiments with Serratia marcescens, atomized from distilled water, from 0.85% NaCl solution, from 0.02% silicon oil emulsion, from 0.1% powdered skim milk suspension, and from 7% meso-Inosit solution. In each case, we filtered for 2 minutes with a suction output of 12 l/minute. For space reasons, we did not show the individual measurement values in this illustration. The yield computation is the same as for Tables 1 and 2.

Key: a. Powdered skim milk
b. Silicon oil
c. Yield
d. Time interval between aerosol filtration and dissolution of filters.

According to electron-microscope investigations (Preusser and P-tras, as yet unpublished), the protective effect of albumin and silicon oil obviously is based on the fact that these substances are deposited in the form of more or less dense films along the surfaces of the atomized bacteria cells. Cooking salt, on the other hand, seems to protect the cells on the basis of its hygroscopic properties. Figure 6 shows that, in aerosol duct experiments, with Serratia marcescens, the colony yield achieved with the help of the Kruse membrane filter method increased as the NaCl content of the atomized suspension increased, until it dropped again at NaCl concentrations of more than 1.7% due to a considerable enlargement of the individual aerosol droplets; this is due to the fact that the enlargement of the droplets resulted in a reduction in the number of impacting droplets. The fact that the droplets became larger as the NaCl concentration increased could clearly be recognized in carbolerythrosin-stained and immersion oil-soaked membrane filter preparations, under the microscope.

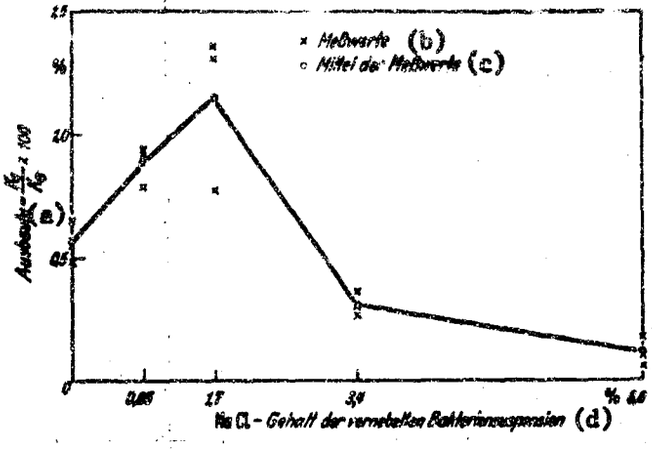

Figure 6. Colony yield when NaCl content of atomized bacteria suspension increases. Aerosol duct experiment with Serratia marcescens. In each case we filtered for 2 minutes (12 l/min) on MF 30 membrane filter with grid. K_o = number of atomized cells capable of multiplying per liter of air, K_1^o = number of cells isolated through filtration at the duct exit and capable of reproducing, per liter of air.

Key: a. Yield
b. Measurement values
c. Average of measurement values
d. NaCl content of atomized bacteria suspension

In order to get indications on the numerical ratio between cells that are capable of reproducing and those that are not capable of reproducing, air germ preparations from the rabbit hutch of the Aerobiology Institute and preparations from Serratia marcescens cells, isolated from the aerosol duct, were incubated as usual for a short time (mostly 4-8 hours) on membrane filters and they were then stained, made transparent, and investigated microscopically. The result of these investigations was unsatisfactory. They were therefore discontinued after some time. A microscopic inspection of preparations of the hutch aerosol, illustrated through filtration according to Kruse, frequently revealed large dust particles to which adhered numerous microorganism cells which could not have come into contact with the nutrient medium during incubation. A decision as to the multiplication [reproduction] capability of such cells was therefore impossible from the very beginning. In preparations illustrated with the help of the gelatin filter method the error caused by such effects appeared rather insignificant but the count of all of the individual cells -- in the evaluation of aerosol duct experiments and in the dissolution of the filters with alcohol and ether or with methylcellosolve -- could be made hardly with the same accuracy as the count of slide preparations.

In other experiments, an attempt was made to modify the air germ isolation method described by Machala and Spurny (1959). The methodology here involved the following: atomized cells of Serratia marcescens were

absorbed from the aerosol duct on membrane filter MF 30 and were t. n suspended in methylcellosolve while the filter material was dissolved. The resulting suspension was then filtered through Cellafilter provided by the Membrane Filter Company ([9] Cellafilter, medium, thoroughly cleaned in sterile distilled water and then soaked in methylcellosolve). Then the Cellafilters were subjected to follow-up treatment by means of the filtration of pure methylcellosolve and 0.85% NaCl solution and they were finally placed on a nutrient medium and incubated. This method turned out to be relatively awkward and vulnerable to error, particularly since the Serratia cells were sensitive to methylcellosolve treatment.

Discussion

In contrast to the impinger methods, which do not facilitate a differentiation between "genuine" air germs and locally occurring accompanying organisms which rapidly die in the atmosphere, the gelatin filter method thus proves to be a rather efficient method for the selective enrichment of the typical components of the air microflora. At the same time it is superior to the impinger methods also because of its higher efficiency; here, investigations are to be performed directly on microorganism preparations isolated from the atmosphere, without the cultivation method as such.

The methods of laboratory evaluation of air germ preparations undoubtedly can be further improved. Jannasch and Jones (1959) described two cultivation methods with whose help they found 20 and 35 times greater live-cell counts in the preparations of marine bacteria than in macro-colony counts. Direct cell counts in their investigation material even yielded 150 times the count for membrane filter preparations and about 2 times the count for slide preparations. We are certainly not hasty in concluding or assuming here that higher cell counts can be achieved also in aerobiological investigations if the laboratory methodology is further developed.

Direct and possibly automatizable germ count methods, which enable us to make a distinction between cells that can multiply and those that cannot multiply, unfortunately are not yet available. Staining methods, such as they were described by Strugger (1949) and by Kuznetsov (1958), cannot be considered for air germ analyses because no autolysis phenomena can be registered in the case of air germs. The same applies to UV-microspectrophotometric methods (see Petras and Ullrich, 1965). Just exactly up to what point micro-colony counts are meaningful (see Wlodawer, 1963) is something that must still be determined. Good success -- although requiring a relatively large expenditure in terms of work -- seems to be indicated by electron-microscope investigations: Preuster and Petras (so far unpublished) discovered in aerosol duct experiments that cells of Serratia marcescens and E coli, after atomization from distilled water and from 0.85% NaCl solution, almost throughout revealed clearly recognizable plasma shrinkages, whereas cells atomized in the presence of skim milk albumen or silicon oil, did not essentially differ, in terms of their internal structure, from similar organisms, coming from culture solutions.

Summary

1. Gelatin filters are considerably more suitable for air germ analyses, generally speaking, than membrane filters. This is based primarily on their water solubility which makes it possible to produce any desired dilutions of the preparations.

2. The vitality of microorganisms, which are in a position to withstand longer stays in the atmosphere and come out alive, is obviously not at all impaired or reduced only very little due to drying and impact effects during the filtration process (filtration perio investigated up to 30 minutes, filtration speed up to at least 60 cm · sec^{-1}).

3. Gelatin filter preparations of such microorganisms can be preserved dry for many days at room temperature, prior to laboratory evaluation, without any essential impairment in the quantitative analysis.

4. In aerosols produced by means of artificial atomization of Bac subtilis var niger (Bac globigii), it is possible to achieve higher yields with the help of the gelatin filter method than when we use the AGL-30 impinger and the impinger according to Bronn.

5. Cells of Serratia marcescens, which normally quickly lose their reproduction capability in case of artificial atomization, tolerate the filtration process and preservation on dry gelatin filters rather poorly. Both as aerosol components and on gelatin filter surfaces they remain intact relatively long, if they are atomized in the presence of certain substances that protect them.

6. The efficiency of the gelatin filters currently made by the Membrane Filter Company for aerosols of Bac subtilis var niger made by atomization from 0.85% NaCl solution is between 99.90% and 99.98%, at filtration speeds of 1-12 cm · sec^{-1}. In the filtration of aerosols with very high moisture contents, the efficiency of the filters can be reduced due to the enlargement of the pores.

7. The gelatin filter method makes it possible to separate the components with a long survival rate from the air microflora and to distinguish them from accidentally occurring short-lived accompanying organisms. At the same time the employment of the gelatin filter method is advisable wherever optical and electron-optical investigations are to be undertaken directly on the isolated air germ material. In this respect it is normally superior to the impinger methods, particularly for the reasons mentioned under points 3, 4, and 6, above, and because of the fact that it can also be used in connection with minus temperatures.

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