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Adherence of Bacteria, Yeast, Blood Cells, and Latex Spheres to Large-Porosity Membrane Filters

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Strong adherence of bacteria, yeast, erythrocytes, leukocytes, platelets, spores, and polystyrene spheres to membrane filter materials was noted during filtration through membranes with pore size diameters much larger than the particles themselves. Quantitative recovery on the membrane filters of these particles from low-concentration suspensions was achieved during gravity- or vacuum-assisted filtration through membranes with pore diameters as much as 30 times that of the filtered particles. Mechanical sieving was not responsible. The phenomenon was judged to be electrostatic. It could be partially blocked by pretreating the filter with a nonionic surfactant (Tween 20), and elution of adherent particles was achieved with 0.05% Tween 20. Gram-positive cocci were removed from suspension more efficiently than gram-negative rods. The commonly used cellulose membranes adsorbed more bacteria, blood cells, and other particles than did polycarbonate filters. Of lesser adsorptive capacity were vinyl acetate, nylon, acrylic, and Teflon membranes. Backwashing with saline, serum, 6% NaCl, dextran solutions, or phosphate buffers of varying molality and pH removed only a fraction of adherent particles. Tween 20 (0.05%) eluted up to 45% of adherent particles in a single back-filtration. Selected filters quantitatively removed the particles tested, which then could be washed and subjected to reagents for a variety of purposes. It is important to anticipate the removal of particles during membrane filtration, since it is not a simple mechanical event.

Particle retention on membrane filters with pore sizes larger than the particles filtered has been reported for viruses as early as 1965 by Cliver (2, 3), who encountered enterovirus retention on cellulose nitrate filters in the pore size range from 50 to 220 nm. Lodish and Zinder (7) noted the attraction of membrane filters for bacteriophage. Nirenberg and Leder (8) utilized membrane filters to attract and hold bacterial ribosomes to supply the necessary components for protein synthesis.

Viruses were concentrated from water and sewage by adsorption to membrane filters (1, 5, 9). Tobin and Dutka (10) found avid heavy-metal ion retention by cellulose filters. By scanning electron microscopy they observed wide variation in the physical appearance of the filter's tortuous fiber maze, dependent on the company of manufacture. Hawker and Hawker (6) reported protein losses during sterilization by filtration. Daniels (4) reviewed the general subject of microbial adherence to solid surfaces.

Particle adherence on membrane filters with pores sizes larger than the particles is of concern in almost all applications of filter technology. For example, there is an option of using large-porosity filters in microbial water analysis in place of 0.22-, 0.45-, or 0.6-μm-pore size filters, to either avoid or delay clogging. Versatility of the filtration process may be increased further when coupled with elution of adherent microbes from the filters for other manipulations such as dilutions for plate counts of colonies, culture on special media, or automated water analysis techniques.

Particles smaller than the pore size of the filter, which one would expect to find in the filtrate, are quite likely to remain, firmly adherent, on the filter. The membrane adsorption effect at best is a quantitative particle collection system. This report is concerned with adsorption of bacteria, spores, yeast, blood cells, and latex spheres to commercial membrane filters.

MATERIALS AND METHODS

Filter apparatus and membranes. All filters were 47 mm in diameter. The pore sizes ranged from 1.0 to 14.0 μm.

The filter membranes were used from the packages, without sterilization. The level of contamination was

low, usually undetectable under the experimental conditions used. Steam or gas sterilization was avoided because of possible effect on pore size and charge characteristics. The polycarbonate filters are only 0.1 as thick (10 μm) as cellulose filters (100 μm). They can be folded and creased without fracturing and are very resistant to ripping. Care was required, however, while twisting the upper and lower filter bodies together, that the filter membrane did not twist and fold. To protect the membrane from these twisting forces, a coarse, thick, cellulose prefilter (Millipore AP-100) was used over the polycarbonate filter. This supporting filter did not retain bacteria.

The autoclavable polycarbonate filter holder used throughout was the 47-mm-diameter Sterifil aseptic system (Millipore Corp., Bedford, Mass.), equipped with a 250-ml receiver flask.

Bacteria. A suspension of Pyrex glass particles, lot 5D, developed for standardization of *Haemophilus influenzae* vaccine, having 10 opacity units at 530 nm and 9 opacity units at 420 nm light wavelength, was kindly supplied by Margaret Pittman.

Strains of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were subcultured daily on nutrient blood agar. Initial suspensions were made in saline from 1-day-old blood agar growth, and these were adjusted to the turbidity of the opacity standard, as judged by eye against natural light through a window. Suspensions of *Bacillus subtilis* spores (Difco) were prepared for filtration in the same way. This provided a stock suspension of between 10^8 and 10^9 colony-forming units (CFU) per ml. Subsequent dilutions were made in Trypticase soy broth (TSB) (BBL Microbiology Systems, Cockeysville, Md.). For most experiments this stock suspension was diluted to provide a high-CFU suspension of about 10^6 CFU/ml and a low-CFU suspension of about 10^3 CFU/ml. In all experiments, 50 ml of suspension was the standard volume filtered through the 47-mm-diameter membrane filters. Dilutions for colony counting were always 10-fold. Plating for CFU counts was done by pipetting 0.1 ml of suspension to the surface of a blood agar plate. This inoculum was spread with a bent glass rod that was stored in 70% ethanol and flamed before use.

To determine whether the filters had a saturation point, eight 50-ml volumes of the same bacterial suspension were filtered, in order, as each filtration was complete. A sterile side-arm flask was used for each successive filtration, and CFU were determined for each filtrate. This procedure was performed on cellulose membranes of 8.0-μm porosity.

Blood cells. Washed human erythrocytes, lymphocytes, and platelets were tested for adsorption to membrane filters after suspensions were made in buffered saline. Cells were counted in a hemacytometer chamber before and after filtration, using Nomarski interference phase optics.

Polystyrene spheres. These very uniformly sized particles were 0.5 μm in diameter and were obtained from Particle Information Services, Inc., Grant's Pass, Ore. Particles were diluted in saline, and particle counting was done in a Petroff-Hausser bacterial counting chamber using Nomarski interference phase optics.

Backwashing (elution). Filter membranes with impinged particles were reversed, placed in an identical sterile filter apparatus, and backwashed by gravity flow with the following: saline; 6% NaCl; serum; dextran solutions (1, 5, 10, 25%); phosphate saline buffers at pH 5.0, 6.0, 7.0, 8.0, and 9.0; and 0.05% Tween 20 (polyoxyethylene sorbitan monolaurate) in neutral phosphate saline buffer. The filters were also eluted in position without reversal.

Scanning electron microscopy. Bacteria on the filters were first fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer, followed by 1% osmium tetroxide. Fixation was necessary to prevent cell distortion during the coating process. Pieces of filter with adherent particles were gold-palladium-coated to 300-Å (30-nm) thickness in a vacuum evaporator with a sample rotation-gyration device, and viewed in a Hitachi Hi-Scan scanning electron microscope in the secondary electron mode.

RESULTS

Retention of the various particles on filters is recorded in Table 1. *S. aureus* and *C. albicans* adhered to the filters much more than did *E. coli*. Cellulose ester filters retained more bacteria than did any other filters. In general, the retention capacity of polycarbonate membranes ranked second. Teflon filters did not retain *E. coli*, but 10-μm pore diameter Teflon filters retained 51% of low-CFU *S. aureus*. Larger particles were retained by the Teflon filters, except for platelets, of which only 16% were retained. The single nylon filter studied, even with a 14.0-μm pore size, retained 76 and 91% of the high and low concentrations of *S. aureus*, respectively, and 28 and 44% of the high and low concentrations, respectively, of *E. coli*. More *C. albicans* cells were adsorbed than those of *S. aureus* or *E. coli*. The pore size that mechanically retains erythrocytes is difficult to predict because of erythrocyte plasticity. Membranes of pore size below 5.0 μm retained all erythrocytes in the suspensions tested. Retention of erythrocytes on other membranes was as follows: 68% on 8.0-μm and 74% on 12.0-μm polycarbonate; 99% on 10.0-μm polypropylene; 99% on 14.0-μm nylon; and 83% on 10.0-μm Teflon.

Retention of lymphocytes on membrane filters is described in Table 1. It is possible that large blood cells are mechanically retained, especially lymphocytes and platelets, which have irregular surfaces. Platelets, however, were remarkably little attracted to filter surfaces of appropriately large pore sizes.

Adherence of platelets to cellulose filters was marked, with 92% percent adsorption on the 8.0-μm membrane. The percentages of platelets retained on other membranes tested are shown in Table 1. Except for the cellulose filters, where retention of platelets may have been related to

the labyrinthine quality of the filter and the rough platelet surface, rather than to charge, there was little adhesion. Moderate retention on various 5.0-μm filters probably indicated mechanical sieving.

Large numbers of saline-suspended polystyrene latex spheres (0.5 μm in diameter) adhered to all of the filters tested. All of the cellulose filters retained more than 90% of these particles. The polycarbonate filters retained 83 to 99%, and the rest of the filters retained more than 90%, except for 80% retention by 10-μm Teflon.

The results of attempts to saturate the holding capacity of high-porosity filters for *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Corynebacterium diptheriae*, *B. subtilis*, and human blood platelets showed evidence of a gradual reduction in retention of particles by the filters; there was evidence for a drop demarcating a saturation point. The suspended particles in eight separate 50-ml volumes partially clogged the filter, even though increasing numbers of particles appeared in successive filtrates.

Elution with 6% NaCl, serum, dextran, and phosphate buffers generally resulted in recovery

TABLE 1. *Percent retention of bacterial and other particles on large-porosity membrane filter materials*

Filter material	Pore size (μm)	Particle concn[a]	% Particles retained on filter						
			E. coli	*S. aureus*	*C. albicans*	Erythrocytes	Lymphocytes	Platelets	Latex spheres (0.5 μm)
Cellulose[b]	1.0	High	100	100	100				96
		Low	100	100	100				
	1.2	High	97	100	100				91
		Low	98	100	100				
	3.0	High	94	100	100				98
		Low	100	100	100				
	5.0	High	78	100	100	100	100	98	95
		Low	98	100	100				
	8.0	High	57	100	100	100	98	92	98
		Low	100	100	100				
Polycarbonate[c]	2.0	High	71	54	100			96	99
		Low	99	65	100				
	3.0	High	66	65	100			76	94
		Low	99	68	100				
	5.0	High	64	71	60	100		24	96
		Low	75	73	79				
	8.0	High	67	77	85	68	71	5	87
		Low	78	81	100				
	10.0	High	17	71	44	78	65	3	88
		Low	26	71	79				
	12.0	High	12	77	10	74	51	3	83
		Low	16	81	17				
Acrylic, polyvinyl chloride, nylon[d]	3.0	High	57	72	100				95
		Low	71	81	100				
	5.0	High	71	67	100			57	93
		Low	98	73	100				
Polyvinyl chloride[b]	2.0	High	88	100	100				93
		Low	93	100	100				
Polypropylene[d]	10.0	High	9	24	75	99	42	11	80
		Low	28	71	79				
Nylon[b]	14.0	High	28	76	100	99	76	19	96
		Low	44	91	100				
Teflon[b]	1.0	High	30	65	100				100
		Low	70	94	100				
	5.0	High	0	56	100	99		54	93
		Low	0	60	100				
	10.0	High	0	44	67	83	54	16	80
		Low	0	51	79				

[a] High concentration, 10^6 particles per ml; low concentration, 10^3 particles per ml.
[b] Millipore Corp. The cellulose filters are described as cellulose esters.
[c] Nuclepore Corp.
[d] Gelman Co.

of 1 to 15% of the various particles. This was probably due to mechanical disruption of particles rather than charge alteration on the filters and particles. Tween 20 (0.05%) eluted three to six times as many particles from 8.0-μm cellulose filters as did buffer alone, indicating charge neutralization activity. Washing of the 8.0-μm pore size cellulose ester membrane with 0.5% Tween 20 just before filtration of *S. aureus* suspensions resulted in a 2.5- to 5-fold reduction in bacterial retention, compared to untreated membranes. Scanning electron microscopy showed close adherence of the particles to filter membranes. Particles were more easily visualized on the polycarbonate than on the cellulosic filters, because on the latter they were distributed along the fibers deep in the filter. Figures 1 and 2 show the 8.0-μm pore size cellulose filter before filtration, and Fig. 3 and 4 show the same size filter after filtration of *E. coli* and latex spheres. Figures 5, 6, 7, and 8 show latex particles, *S. aureus*, and *E. coli* on polycarbonate filters. Processes apparently derived from the bacterial cell walls are seen in Fig. 7 and 8, extending to the filter surface. Particles of any nature usually lined the cylinders of the polycarbonate pores. There was evidence of a scouring effect of the liquid during filtration (Fig. 6), as the rims of the pores are bare of *S. aureus* cells. All of the particles occurred only in monolayers. The filter became saturated when most of the available surface was covered with a single layer of particles (Fig. 6).

DISCUSSION

The adherence of particles to membrane filters with pore sizes larger than the particles should be of concern in all applications of filter technology. For example, there is an option of using large-porosity filters in microbial water analysis in place of 0.22-, 0.45-, or 0.6-μm-pore size filters, to avoid or to delay clogging. Versatility of the filtration process may be increased further when coupled with elution of adherent microbial particles for other manipulations such as dilutions for plate counts of colonies, culture on special media, or automated water analysis techniques.

Particle retention as described here is not usually sterilizing, and no tests were done to determine sterility. Expression of 100% retained bacteria was based on plating of 0.1 ml of undiluted filtrate. It is possible that a few colonies would be detected if 1.0 ml or more of these filtrates had been plated. When the purpose of filtration is sterilization, selection of filters with pore sizes of 0.2 to 0.45 μm is appropriate. But for the preliminary removal of filter-clogging particles, the appropriate large-pore filter could be utilized successfully. The total holding capacity of the filters is striking. Industrial filtration processes can obviously make use of the phenomenon. The adsorption technique, as used for viruses, is lethal to bacteria. The requirement for extremely low pH (3.5) for adsorbance, and high pH (11.5) for elution, precludes the use of this technique for bacterial recovery. Also, experiments conducted in this laboratory indicate that elution with buffers of bacteria and other relatively large particles from membrane filters is far less quantitative than reported elution of viruses or proteins. With the use of multiple elution volumes or of recirculation with 0.05% Tween 20, approximately 100% particle recovery was easily achieved. Upward of 50% elution was possible with a single elution volume, with no adverse effect on the organisms.

Bacteriophage filtration through 0.22- or 0.45-μm membranes results in a 10- to 1,000-fold loss of phage plaque counts. Attempts to reduce this loss by prewashing the filter with broth or serum solution were minimally successful. There is advantage to be taken of the phenomenon in many areas of bacterial analysis, however. It permits filtration of larger volumes of liquids than would otherwise be possible without filter clogging, to achieve just a little less than absolute removal of selected particles. The application of the phenomenon in bacterial recovery analysis deserves further study. Certainly optimal bacterial recovery will occur where there is minimal competition for filter binding capacity from extraneous particles. Bacteria are adsorbed more efficiently as the number of CFU per milliliter is reduced, although the adsorbance capacity may be unchanged. This bodes well for applications where few suspended organisms are adsorbed such as, for example, a lysis-filtration blood culture technique (11).

As pore size increases in a membrane series, there is reduced adsorbance that should not occur through a strictly mechanical consideration. The best example of this is the 0.5-μm-diameter polystyrene particles and the polycarbonate membranes, which showed 99% adsorbance on the 2.0-μm membrane, ranging down to 83% adsorbance on the 12.0-μm membrane. This is probably because the distance from the particle to the wall of the pore becomes greater than the diameter of the particle, reducing charge attraction below that required to divert the particle to the membrane.

Vacuum filtration of 50 ml of suspension through large-pore membrane filters requires about 4 to 5 s. The flow rate and the adsorptive action of the filter material for the suspended particles are rapid. Retention during vacuum filtration of the particles tested was about the

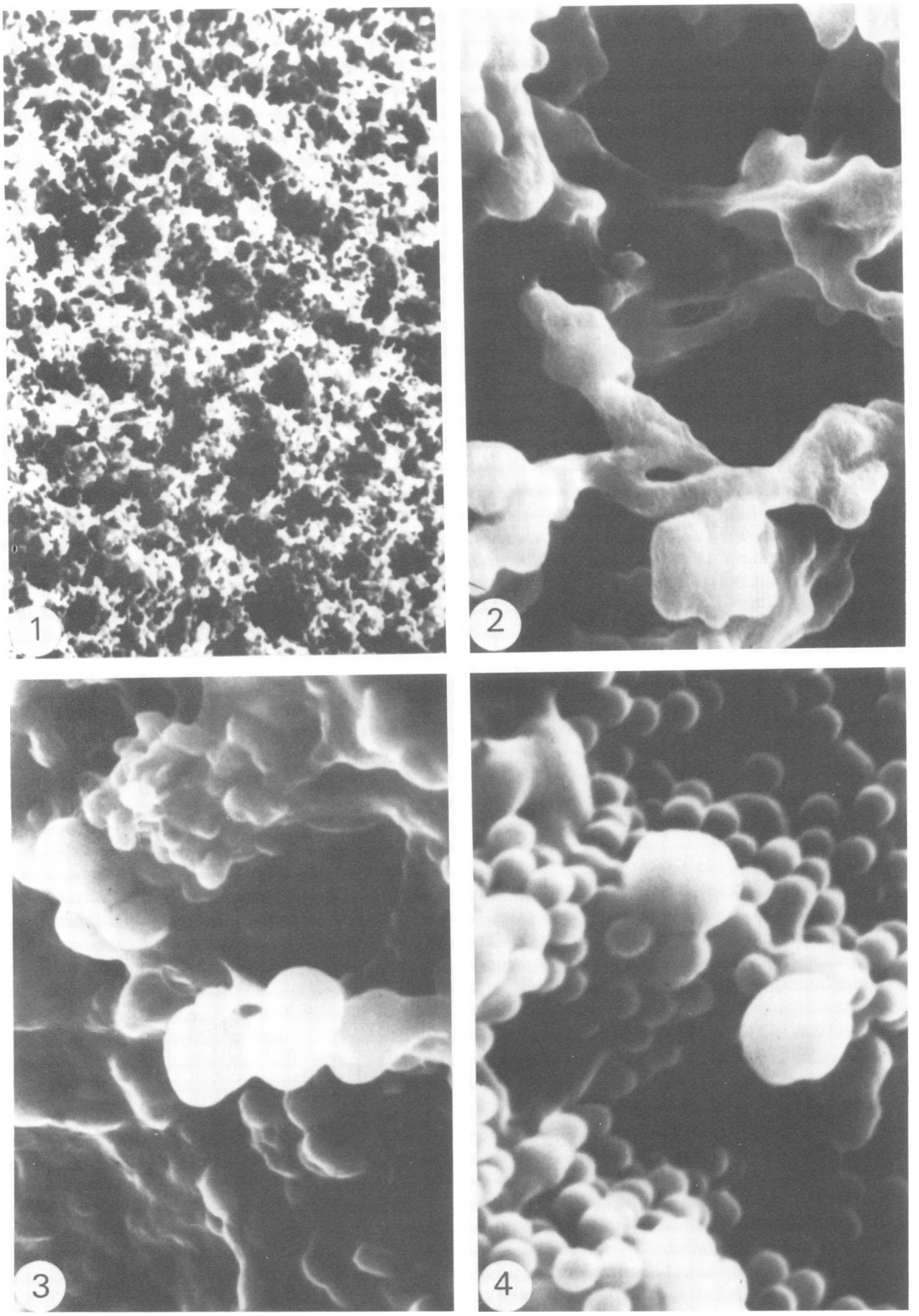
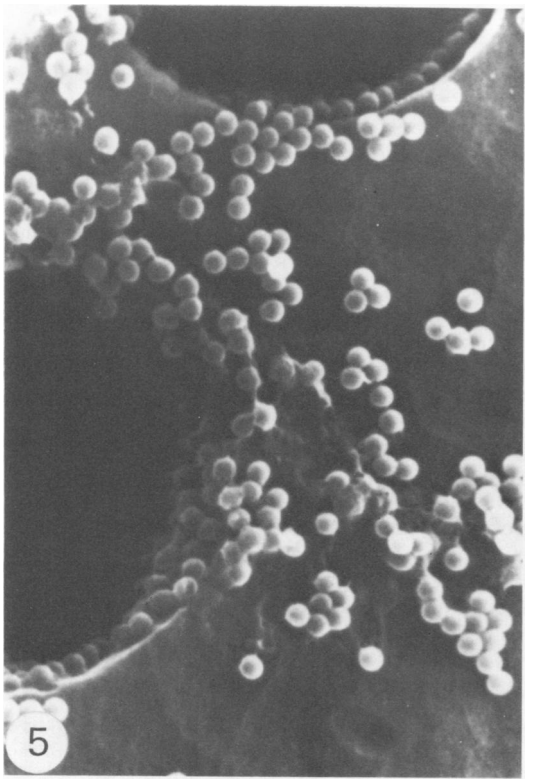


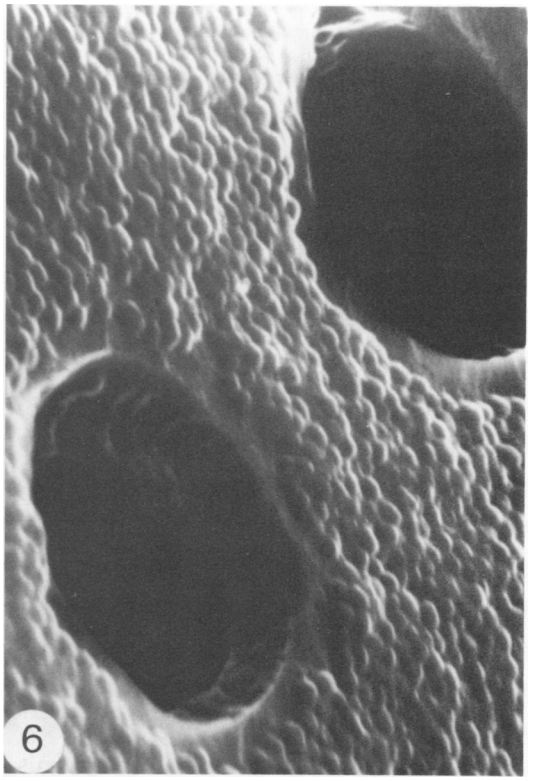
FIG. 1. *Cellulose ester solvent cast membrane filter (Millipore); 8.0-μm pore size; ×1,000. Scanning electron micrograph.*

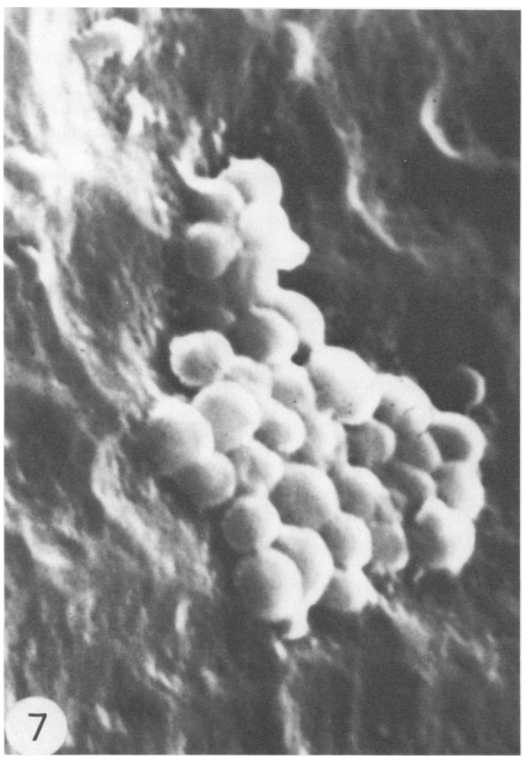
FIG. 2. *Cellulose ester solvent cast membrane filter (Millipore); 8.0-μm pore size; ×10,000. Scanning electron micrograph.*

FIG. 3. *Cellulose ester solvent cast membrane filter (Millipore); 8.0-μm pore size; with closely adherent E. coli cells. Compare with Fig. 2. ×5,000.*

FIG. 4. *Cellulose ester solvent cast membrane filter (Millipore); 8.0-μm pore size; showing adherent 0.5-μm-diameter acrylic latex spheres collected during filtration. The large white spheres are the clubbed ends of filter strands. ×5,000.*







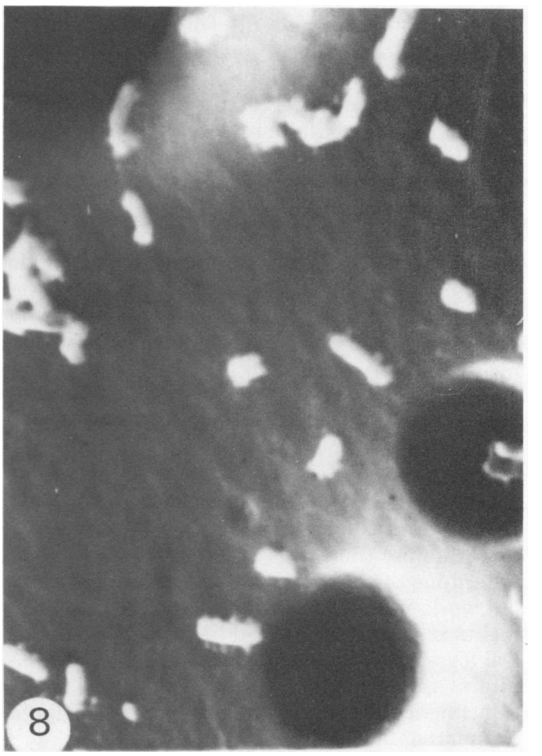


FIG. 5. *Polycarbonate membrane (Nuclepore), 12.0-μm pore size, with 0.5-μm acrylic latex spheres. Latex particles thickly coat the cylindrical pores as well as the top surface. ×5,000.*

FIG. 6. *Polycarbonate membrane (Nuclepore), 12.0-μm pore size, showing tightly packed, adherent S. aureus cells. ×5,000.*

FIG. 7. *Polycarbonate membrane with attached S. aureus cells. Attachment is very firm, with evidence of a matrix, perhaps cell wall derived, between filter and bacterial cells. ×5,000.*

FIG. 8. *Polycarbonate membrane, 12.0-μm pore size, and attached E. coli cells. As with S. aureus, there is evidence of microprocesses between organism and filter. ×3,000.*

same as that during gravity filtration, which required approximately 2 to 4 min.

Saturation data indicate a bacterial load maximum (as a monolayer) of about 10^5 to 10^7 CFU per filter (8.0-μm, 47-mm-diameter) for the two bacterial species tested. The diminishing percent retention in successive filtered samples starts at these approximate values. There is no sharp reduction in membrane-attached particle saturation for the relatively simple polycarbonate filters, nor for the complex and tortuous maze of the cellulose filters. The surface area of polycarbonate filters is essentially that of the plane surface, less that area taken up by the filter holding apparatus. Total area of the 47-mm filter is 17.3 cm^2. The polycarbonate filter is a 10-μm-thick plastic film, with punched out holes formed by high-energy particle bombardment followed by chemical etching. The cellulose or solvent cast filter is 100 to 150 μm thick, 10 times that of the polycarbonate. Moreover, its configuration of tangled, convoluted strands greatly increases the total surface area, far above that of the corresponding polycarbonate filter.

Measurement of charge in arbitrary units using a sensitive electrometer indicated that all solvent cast membrane filter materials such as Teflon, nylon, polycarbonate, cellulose, etc., have strong surface charges, whereas ordinary fibrous cellulose materials that are not solvent cast, such as the Millipore AP4700 prefilters, do not. Increasing charge with a high voltage generator off a bronze brush surface did not change particle retention characteristics of the filters. The surface charge, as measured in air, probably is not active in particle attraction from liquids (liquid immersion). Reversing the polarity of the high-voltage generator and thereby reversing the surface charge on filter membranes had no measurable effect on their attractiveness for the various particles. Reducing surface charge with a polonium-210 ionization source (500 μCi) also did not affect particle retention from aqueous suspensions.

Membrane filters are often used for measuring particle size. Conversely, particles of known size, for example, the series of latexes, are used to determine the pore size of filters. From the present work, it is plain that each of these measurements is greatly influenced by the electrochemical forces at work between the particles and the filters. Assumption of a purely mechanical filtration might lead and probably has led to large errors in data interpretation.

Although the adherence propensity of membrane filters through charge effects does not obviate the need for mechanical sieving, it may be necessary to the use of 0.2-μm filters, and more certainly to that of 0.45- or 0.6-μm filters, for sterility filtration. The known presence of at least a few oversized pores in most filter membranes would seem to support this contention. On a strictly mechanical basis, all that would be required for failure of a sterility filtration is one oversized pore. Whether passage of one or a few organisms is then detected during sterility testing depends upon a complex mix of growth capabilities of the species, volume of filtrate cultured, culture medium, etc.

Development and manufacture of special-purpose filter materials with more intrinsic charge than those currently available would extend the usefulness of the phenomenon. Conversely, manufacturing techniques could be developed that build less intrinsic charge into filters to be used when adsorption is not desired.

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LITERATURE CITED

1. **Berg, G., D. R. Dahling, and D. Berman.** 1971. Recovery of small quantities of viruses from clean waters on cellulose nitrate membrane filters. Appl. Microbiol. **22:** 608–14.
2. **Cliver, D. O.** 1965. Factors in the membrane filtration of enteroviruses. Appl. Microbiol. **13:**417–425.
3. **Cliver, D. O.** 1968. Virus interactions with membrane filters. Biotechnol. Bioeng. **10:**877–889.
4. **Daniels, S. L.** 1972. The adsorption of microorganisms onto solid surfaces: a review. Dev. Ind. Microbiol. **13:** 211–253.
5. **Gerba, C. P., S. R. Farrah, S. M. Goyal, C. Wallis, and J. L. Melnick.** 1978. Concentration of enteroviruses from large volumes of tap water, treated sewage, and seawater. Appl. Environ. Microbiol. **35:**540–548.
6. **Hawker, R. J., and L. M. Hawker.** 1975. Protein losses during sterilizing by filtration. Lab. Pract. **24:**805–807, 814.
7. **Lodish, H. G., and N. D. Zinder.** 1965. Attachment of f2 bacteriophage to cellulose nitrate filters. Biochem. Biophys. Res. Commun. **19:**269–278.
8. **Nirenberg, M., and P. Leder.** 1964. RNA codewords and protein synthesis. Science **145:**1399–1407.
9. **Sobsey, M. D., C. Wallis, M. Henderson, and J. L. Melnick.** 1973. Concentration of enteroviruses from large volumes of water. Appl. Microbiol. **26:**529–534.
10. **Tobin, R. S., and B. J. Dutka.** 1977. Comparison of the surface structure, metal binding, and fecal coliform recoveries of nine membrane filters. Appl. Environ. Microbiol. **34:**69–79.
11. **Zierdt, C. H., R. L. Kagan, and J. D. MacLowry.** 1977. Development of a lysis-filtration blood culture technique. J. Clin. Microbiol. **5:**46–50.