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Inhibitory Effects of Detergents in Membrane Filters

S. A. ROSENBLUTH and G. W. CRIPPS

Abstract □ Aqueous filtrates from membrane filters contain material(s) with UV spectral characteristics quite similar to those of a nonionic isooctyl phenoxy polyethoxy ethanol (IPPE)-type detergent. A permeability test utilizing mammalian cell cultures was employed to evaluate the inhibitory properties of the extract and commercial IPPE. The cell culture test was sensitive to IPPE in concentrations as low as 0.006 percent and produced comparable results with extract concentrations having spectral absorbance (283 mμ) values equal to those of IPPE. Dose-response patterns were markedly similar. Spectral analyses of successive aqueous filtrates were used to study the extractability of the offending agent(s). Treatment of membrane filters with hot water prior to autoclaving rendered the filters safe for sterilization of solutions including cell culture media components.

Keyphrases □ Detergents in membrane filters □ Membrane filter extracts, effect—cell cultures □ Cell cultures, mammalian—filter extract inhibition □ UV spectrophotometry—identification

The presence of water-extractable detergents in membrane filters has concerned a number of investigators. Cahn noted persistent foams in solutions which were membrane-filtered and reported reductions in plating efficiency and degree of differentiation in cultured cells when medium was filtered through unwashed membranes (1). He further suggested that this type filter contains 2 to 3% by weight of water-soluble material(s) which may include an isooctyl phenoxy polyethoxy ethanol [1] (IPPE) or similar detergents. Such leached agents might not be detected when media contain solubilized protein (1), when large volumes of filtrate dilute the contaminant (2), or when relatively insensitive methods are employed. Conversely, problems may be anticipated with small volumes of filtered fluids, defined media, and/or sensitive biological and chemical tests.

Membrane filters are routinely used in the authors' laboratories for sterilization of various solutions including tissue culture media components. The purpose of this paper was to: (*a*) establish the patterns of extraction of detergent(s) by repeated filtration of volumes of hot and cold water; (*b*) quantitize certain inhibitory effects to mammalian cell cultures by aqueous

[1] Trademarked as Triton X-100, Rohm & Haas Co., Philadelphia, Pa. The material used in the present study bore the lot number 1984 and was obtained by courtesy of Mr. T. S. Rowland.

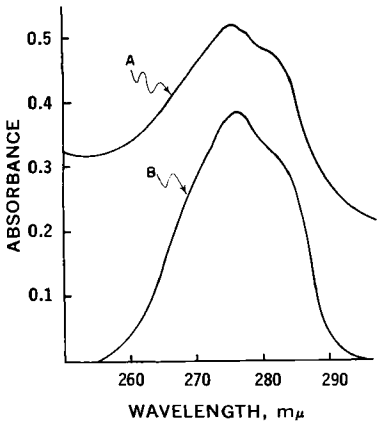

Figure 1—*Ultraviolet absorbance spectra for concentrated aqueous membrane filtrate and an aqueous solution of IPPE. Key: A, membrane extract; B, IPPE.*

filtrates from Gelman membranes and also by commercial IPPE; and (*c*) utilize the experimental data as a basis for pretreating membranes prior to their use in laboratory filtration procedures.

EXPERIMENTAL

Materials—Membrane filters[2] utilized in this study were 47 mm. diameter, 0.2 μ pore size, control number 1146. The filtration system included either a stainless pressure filter holder[3] or a 47-mm stainless steel pressure filtration funnel.[4] A vacuum oven was used.[5] Spectrophotometric analyses were accomplished with a model DU spectrophotometer.[6]

The cultured cell line utilized was Strain L mouse fibroblasts.[7] Growth medium was basal medium (Eagle) containing 10% calf serum in addition to 100 units of penicillin G potassium and 50 mcg. of streptomycin sulfate/ml.[8] During experiments cells were suspended in Dulbecco's phosphate buffered salt solution (PBS) (3).

Methods—*Physical-Chemical*—Utilizing pressure filtration procedures, successive 30-ml. samples of hot (90–100°) or cold (15–20°) water were filtered through individual membranes. IPPE solutions and membrane filtrates were examined for absorbance at 283 mμ as an estimate of detergent concentration (4,5). Dilute solutions were concentrated from 30 to 6 ml. in the vacuum oven at 78°. UV spectra of membrane extracts and IPPE solutions were prepared for comparative purposes.

Cell Culture Studies—Strain L cells were harvested from glass bottles with the aid of trypsin and suspended in phosphate buffered salt solution (PBS). Cell suspensions were diluted to 75–150 × 10^4 cells/ml. and maintained on a magnetic stirrer. Test solutions were graded concentrations of either IPPE or membrane filtrate in PBS. Concentrations of the membrane filtrates were established relative to concentrations of IPPE having equal absorbance values at 283 mμ. One-milliliter aliquots of cell suspension were mixed with 1 ml. of 1:5000 solution of trypan blue in PBS and 0.5 ml. of test solution. Controls received 0.5 ml. of PBS in place of the test solution. Cells were counted microscopically on a hemocytometer 7 min. after addition of test or control solution. Inhibitory effects of the solutions were expressed as percent of cells stained blue.

[2] Methicel Filters, type GA-8, Gelman Instrument Co. The filters were reported by the manufacturer to contain the wetting agent, Poly-tergent G-400, an Olin chemicals product, designated as an octyl phenoxy polyethoxy ethanol.
[3] No. XX40 047 00, Millipore Filter Corp.
[4] Gelman Instrument Co.
[5] Thelco, model 19, Precision Scientific Co.
[6] Beckman Instrument, Inc.
[7] The original inoculum was obtained by courtesy of Carter Lewis, Department of Microbiology, the University of Tennessee.
[8] Microbiological Associates.

Table I—Extraction of Individual Membrane Filters by Hot Water Filtrates

Membrane Number	Absorbance (283 mμ) of 30-ml. Portions after Concentration to 6 ml.[a] — Portion Number — 1	2	3	4	5	6
I	0.180	0.060	0.050	0.065	0.043	0.038
II	0.196	0.065	0.047	0.031	0.022	0.015
III	0.205	0.058	0.040	0.043	0.028	0.015
IV	0.085	0.027	0.022	0.020	0.012	0.013
V	0.092	0.023	0.027	0.020	0.020	0.013
Mean	0.151	0.047	0.037	0.036	0.025	0.019
±*SD*	0.059	0.020	0.012	0.019	0.012	0.011

[a] Individual membrane filters, averaging 52.7 ± 2.5 mg. in weight, were 47 mm. in diameter. Thirty-milliliter portions of hot (90 to 100°) water were successively pressure filtered through each membrane. See text for further details.

RESULTS AND DISCUSSION

Physical-Chemical—Typical UV spectral scans for a concentrated membrane extract and for an aqueous solution of IPPE are shown in Fig. 1. Both exhibit maxima in the region of 275–277 mμ and inflections at 283 mμ, which are characteristics of IPPE (4, 5). Linear relationships between absorbance at 283 mμ and concentration were demonstrated for both materials indicating that Beer's law was obeyed.

Vacuum concentration of extract or IPPE solutions was found to be a convenient means for increasing the sensitivity of the spectral assay. With very dilute solutions however, some low absorbance values were attributed to adsorption on glassware (4). Graded concentrations of IPPE, which were too dilute for analysis, after vacuum concentration, produced absorbance (283mμ) values which were comparable to those represented by the standard plot.

Successive volumes of cold water filtered through membranes extracted detergent poorly and in unpredictable patterns. Hot water filtered individually through five membranes (47-mm. diameter) extracted detergent as illustrated in Table I. The first 30-ml. portions invariably contained the largest quantities of detergent which were three or more times the concentration of the second 30-ml. portions. Subsequent portions contained progressively smaller quantities, with even the sixth sample showing some evidence of the extract. This pattern substantiated Cahn's observation (1) that the detergent was not entirely removed during hot-water filtration and appeared to some extent in subsequent filtrates. Cahn's approaches to protecting filtrates from membrane detergents[9] included discarding initial volumes of the filtrate, prewashing membranes with hot water, and keeping the apparatus and

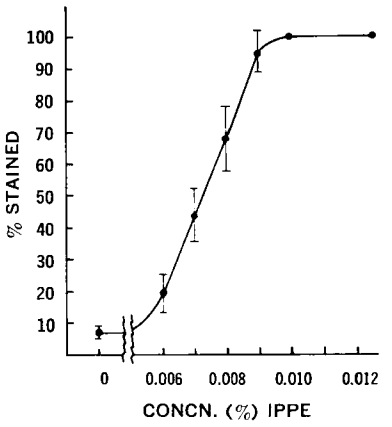

Figure 2—*Percent staining of mammalian cells by trypan blue as a function of IPPE concentration.*

[9] Millipore Filter Corp.

Table II—Comparison of Inhibitory Effects of Aqueous Solutions of IPPE and Aqueous Membrane Extracts

Concn., %	—Percent of Cells Stained by Trypan Blue— IPPE Solutions[a]	Membrane Extracts[b]
0	7.2 ± 1.7	5.8 ± 0.7
0.0030	10.0	—
0.0041	—	15.3
0.0060	19.3 ± 6.4	33.6
0.0069	—	33.2
0.0070	43.8 ± 8.2	42.2
0.0080	67.7 ± 10.4	64.9
0.0090	95.5 ± 6.4	86.2
0.0100	100	—
0.0103	—	100
0.0125	100	—

[a] Data were collected from four experiments. Test solutions were prepared from accurately weighed and diluted IPPE. [b] Data were collected from two experiments. Concentration percentages for the membrane extracts were determined arbitrarily as equal to that of IPPE solutions having the same absorbance (283 mμ).

solution cool prior to and during filtration. The present data suggest these may also be valid methods for the membrane filters used in the present study.[10]

Cell Culture Studies—Figure 2 is a dose-response curve representing the percent staining of cell populations by trypan blue as a function of IPPE concentration. Data collected in a series of four experiments show quite clearly that inhibitory concentrations fell beyond 0.006%. Between 0.006 and 0.010%, staining was proportional to concentration. It is not surprising that the critical micelle concentration for IPPE in water is in the region of 0.01% (4). Further experiments revealed that concentrations of the membrane extract and of IPPE which produced equivalent absorbance (283 mμ) readings, also demonstrated similar inhibitory properties (Table II). Similarities in the spectral and cell inhibition properties of IPPE and the membrane extract were striking and serve to reinforce Cahn's contention (1) that membrane extracts may have included IPPE or a similar detergent.

Filtration of 200 ml. of hot water (1) through individual membranes prior to autoclaving has become standard procedure in this laboratory. There apparently remains a sufficient quantity of detergent to assure wettability of the membrane during sterilization and filtration. However, solutions filtered through such membranes have exhibited no observable tendency to inhibit cultures as compared to unfiltered controls. The danger of contaminating media, laboratory solutions, and even prescription medication with components from membrane filters is apparent. Methods presented here and by Cahn (1) should be helpful in detecting and eliminating such problems. Detergent-free membranes may be obtained on specific request from the manufacturers, but difficulties have been reported in their use (1).

[10] Gelman Instrument Co.

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