Short Communication

THE USE OF MEMBRANE FILTERS IN THE ENUMERATION OF DAMAGED *ESCHERICHIA COLI*

BY N. D. HARRIS AND J. P. RICHARDS

From the School of Pharmacy, Chelsea College of Science and Technology, London S.W.3

Received May 1, 1963

IN a previous communication (Harris and Richards, 1958) the authors showed that *Escherichia coli* cells which had been treated with phenol gave lower counts on membrane filters than on nutrient agar. They suggested that the cause of this discrepancy was either the inability of the membrane to allow diffusion of essential growth requirements, or the presence of toxic materials in the environment of the cells. Gaspar and Leise (1956) reported that *Pasteurella tularensis* was inhibited by the ink used for marking the grids on membrane filters and in the absence of grids the recovery was as good as or better than that on nutrient agar. The effect was not observed with *Pasteurella pestis, Salmonella typhi* or *Vibrio comma.*

Therefore, the possible effects of the grid imprint material on the recovery of phenol-treated *E. coli* have been investigated. Further, the possibility that the membranes contain other toxic materials has been studied and membranes from three different manufacturers have been compared for their ability to cultivate phenol-treated *E. coli.*

The materials and methods employed were those described by Harris and Richards (1958) with the following additions.

Organism and its treatment with phenol. Only one strain of *E. coli* (type I, 44°+, IMViC++−−) was used, namely strain II of Harris, Richards and Whitefield (1961).

The growth from a 24 hr. agar culture, suspended in distilled water, was exposed to 1 per cent w/v of phenol to give a mortality greater than 90 per cent, and survivors were counted after diluting 10^4 times in distilled water.

Membrane filters and counting technique. Filters (8 cm. diameter) from three different manufacturers were used; Courtaulds Ltd. (Oxoid) with and without the grid imprint but from the same batch; Millipore Filter Corporation, Hydrosol Assay (HA) grade white Millipore filter with grids; Membranfiltergesellschaft (MFG), Coli 6 grade without grid markings. When washed membranes were required these were boiled gently with 100 ml. of distilled water, dried and sterilised as usual. In some experiments the filter washings were incorporated in nutrient agar so that 20 ml. of nutrient agar contained the washings from a single filter.

The filters were sterilised by autoclaving, dried overnight at 40°, and supported for counting either on Whatman No. 17 filter pads which had been treated similarly or, less often, on nutrient agar.

The nutrient broth used contained 16 g./litre of "Oxoid" Lab-Lemco granules (CM 15) and was set with 15 g./litre of New Zealand agar when

required. All counts were done by the surface viable method using 10 replicate drops and incubating for 24 hr. at 37°.

Results and Discussion

Effect of grid markings. Both untreated and phenol-treated cells were counted on nutrient agar and on membrane filters with and without the grid markings. Using untreated cells no differences in the counts were observed under the different conditions. The results with phenol-treated cells are presented in Table IA, which shows that very poor recoveries were

TABLE I

VIABLE COUNTS OF PHENOL-TREATED *E. coli* ON NUTRIENT AGAR AND ON OXOID MEMBRANE FILTERS

A. The effect of printed grids			B. The effect of washing the filters		
Mean viable count/drop			Mean viable count/drop		
Nutrient agar (control)	Membrane filters		Nutrient agar (control)	Membrane filters	
	With grid	Without grid		Unwashed	Washed
52·8	18·6	—	20·7	—	7·4
67·8	24·3	—	67·6	—	31·6
86·1	27·7	—	61·3	17·7	30·5
54·4	22·7	—	85·1	38·2	33·2
88·1	56·5	—	4·1	0·1	0
121·0	63·4	50·2	82·2	21·2	31·6
114·5	80·3	65·9	92·3	51·1	47·6
65·9	33·0	31·7	125·0	55·9	64·7
125·7	49·4	61·7	20·8	3·9	3·1
86·8	59·4	34·9	27·5	6·1	8·8
19·9	3·0	2·0			
127·3	96·0	47·7			
100*	48*	41*	100*	30*	36*

*Mean response (per cent of control).

obtained on the membrane filters, thus confirming the results of Harris and Richards (1958). Both types of membrane filter gave significantly lower counts than did the nutrient agar (P, <0·001 in both cases). The membrane filter without the grid markings usually gave slightly lower counts than the membrane with the grids but the differences were not significant ('*t*', 2·13; d.f., 6; P, 0·05–0·1). Thus the printed grids were not implicated in the low counts obtained on the membrane filters.

Tests for the presence of toxic materials in the membrane filters. The possibility that water soluble toxic materials are present in the membrane filters was investigated using washed membranes. Untreated cells cultured on nutrient agar and on the membrane filters gave no differences in count. The results using phenol-treated cells are presented in Table IB. Both washed and unwashed membrane filters gave significantly lower counts than did the nutrient agar (P, <0·001). The washed membrane filters gave a slightly higher mean count than did the unwashed ones, but the difference was not significant ('*t*', 1·02; d.f., 7; P, 0·3–0·4). The inclusion of washings in nutrient agar did not influence the viable counts of phenol treated cells. Thus any inhibitory factor, if present, either was not water soluble or did not diffuse freely out of the membrane filter and so was

concentrated in it, in the immediate environment of the damaged cells. One difficulty in the interpretation of the results with washed membrane filters was caused by the failure of the drops of inoculum to spread as they did on unwashed filters, and the influence of this on the counts cannot be determined.

TABLE II

VIABLE COUNTS OF *E. coli* ON THREE DIFFERENT TYPES OF MEMBRANE FILTERS AND ON NUTRIENT AGAR

Treatment	Mean viable count/drop						
	Nutrient agar (control)	Membrane filters					
		Oxoid		Millipore		MFG	
		N.A.**	F.P.**	N.A.	F.P.	N.A.	F.P.
None	35·8	32·1	33·3	—	—	—	—
	40·8	36·8	39·8	35·6	25·0	33·4	21·5
	39·1	36·5	32·3	34·7	25·9	36·4	35·7
	34·2	36·1	33·1	33·6	31·3	35·0	31·0
	100*	95*	93*	91*	73*	92*	78*
Phenol	205·0	—	—	83·0	70·3	—	84·0
	215·2	145·4	—	90·2	26·9	88·4	—
	204·6	134·4	—	83·8	34·3	93·8	48·7
	128·4	73·0	33·0	—	3·1	—	1·8
	210·8	110·6	—	80·6	53·6	81·8	43·7
	136·6	94·4	—	62·1	24·7	53·2	12·9
	141·6	83·6	—	56·1	12·5	42·1	14·1
	16·9	1·3	0·4	0·3	0·4	0·4	0·3
	100*	54*	14*	33 *	15*	33*	15*

*Mean response (per cent of control).

**Membrane filters incubated on: N.A., nutrient agar; F.P., No. 17 filter paper pad impregnated with broth.

Comparison of three different types of membrane filter. Untreated and phenol-treated cells were cultivated on nutrient agar and on the three different membrane filters, the filters being used both on the surface of nutrient agar and on filter paper pads, and the results are given in Table II. Using untreated cells none of the counts was significantly different from the nutrient agar control. In contrast with these results, Hess and Speiser (1959) using MFG Co 5 membrane filters observed that counts from dilute suspensions of *Pesudomonas pyocyanea* were significantly higher than with pour plates, though the differences were small.

With phenol-treated cells cultured on the membrane filters there were substantial reductions in the counts (Table II), and both the Millipore and the MFG filters gave even fewer colonies than the Oxoid, whether they were incubated on the surface of the nutrient agar or on the filter paper pads. The differences between the counts on nutrient agar and on the membrane filters were significant in all cases (P, $<0{\cdot}01$).

Thus it is clear that the poor performance of membrane filters relative to nutrient agar reported by Harris and Richards (1958) was not an isolated occurrence with one type of filter only and may be associated generally with the membrane filter technique. In this connection Lightbown (1962) found poor recoveries of *Bacillus subtilis* on membrane filters under some conditions.

Postgate and Hunter (1962) have observed that membrane filters contained substances which accelerated the death of starved bacterial populations, and it was suspected that the membrane filters contained surface-active substances, since drops spread much less on washed Oxoid filters than on unwashed ones. The presence of anionic surfactants in the Millipore and Oxoid membrane filters (but not in the MFG) was confirmed by surface tension measurements on the washings (about 55 to 60 dynes/cm.) and by dye transference tests.

Further, preliminary experiments using suspensions treated with cetrimide (0·005 per cent w/v) showed that counts on unwashed membrane filters were similar to those on nutrient agar, while washed membrane filters gave lower counts. This phenomenon may have resulted from neutralisation of the cetrimide carried over in the inoculum by surfactants present in the filter. The results, however, are insufficiently conclusive to warrant a definite opinion about the reasons for the low counts of cells treated with phenol on the membrane filters. Since membrane filters can give poor recoveries of damaged cells, the use of such filters in sterility testing and similar applications should be approached cautiously.

REFERENCES

Gaspar, A. J. and Leise, J. M. (1956). *J. Bact.*, **71**, 728–731.
Harris, N. D. and Richards, J. P. (1958). *J. appl. Bact.*, **21**, 86–93.
Harris, N. D., Richards, J. P. and Whitefield, M. (1961). *Ibid.*, **24**, 182–187.
Hess, H. and Speiser, P. (1959). *J. Pharm. Pharmacol.*, **11**, 650–658.
Lightbown, J. W. (1962). *Proceedings of the 7th International Congress for Microbiological Standardisation*, pp. 220–225. Edinburgh and London: Livingstone.
Postgate, J. R. and Hunter, J. R. (1962). *J. gen. Microbiol.*, **29**, 233–263.

The paper was presented by DR. RICHARDS.