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The effect of membrane filters on cultured cells

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The use of membrane filters for filtration of serum-containing culture media can change a medium in such a way that cells cultured therein attain a significantly higher lactate-glucose metabolism than the same type of cells cultured in unfiltered medium. Five different filtertypes were examined, but the response was only found after filtration with MF-filters of Millipore (mixed esters of cellulose). Furthermore, in two of the three cell lines examined minor growth inhibitions were also observed. The MF-filters were found to contain a compound bound to the filter material so that serum-containing medium, but not serum-free medium, or water, could extract it in amounts sufficient for metabolic – and growth effects. The compound in MF-filters is closely related to Triton-X-100 both with respect to u.v.-spectra and to biological effects.

During investigations of the metabolism and growth of HeLa cells (Engel & Binderup 1973) it was observed that the level of metabolism of the cells unexpectedly doubled, and the growth of the cells became poorer. Since there was a correlation between these changes and the introduction of an altered sterilization procedure, we decided to investigate the problem further.

METHODS

HeLaSI from Statens Seruminstitut, HeLa CCL 2 purchased from American Type Culture Collection, and Yoshida rat hepatoma cells were cultured in Earles MEM + non-essential amino acids containing double concentrations of MEM vitamins, and 5 or 10% newborn calf serum purchased from Gibco, or produced in the laboratory.

Calf serum was heat-inactivated for 30 min at 60 °C; gentamycin 500 μg ml^{-1}, penicillin, 1000 U ml^{-1}, streptomycin, 500 U ml^{-1}, and neomycin, 74 U ml^{-1}, corresponding to 10 times the recommended concentrations for complete medium (1) were added. Serum was stored at −20 °C until use. Inoculations took place from confluent cultures run in weekly passages. The cells were loosened by moistening with 0·1% trypsin (Sigma) for 5 min at 37 °C, followed by mechanical separation, coulter counting, and even distribution into 80 ml Nunclon flasks (Nunc, Roskilde, Denmark), each containing 40 ml of culture medium. Just before gas tight closure, 1 ml of 1·2% $NaHCO_3$ was added to each flask.

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Our cell lines were examined for mycoplasma injection on three different occasions with negative results.

Sterilization procedures

'Non-filtered' control media were produced by mixing 10 times concentrated, autoclaved Earle's salt solution with aqueous solutions of vitamins and amino acids filtered through 25 mm ø Schleicher & Schüll (S&S) 1120 filter, and calf serum.

'Filtered media' were produced by mixing together all of the components of the media including the serum before filtration through the filter selected for examination took place. The 90 mm ø filter holder contained 1 filter per 200 ml of medium. Before filtration the filters were washed with 500 ml of 0·9% NaCl (saline).

Ethanol-soluble components of membrane filters

A 90 mm, washed, membrane filter was placed in the bottom of a beaker. Ten ml of ethanol was added and stirred occasionally for 10 min. The extract was evaporated to dryness followed by autoclaving. Medium was added to the dry matter and stirred for 30 min.

Analyses

Corresponding values of cell number, lactate (Gutman & Wahlefeld 1974), and glucose concentrations (Schmidt 1961) were determined daily for 3 days during exponential growth in independent flasks. Lactate and glucose metabolism per cell and per hour, of exponentially growing cells, were calculated as described earlier (Engel & Binderup 1973).

DNA was determined according to the method of Burton (1956) with modifications of Leyva & Kelly (1974).

RESULTS AND DISCUSSION

Table 1 demonstrates the effect of MF-filtration on 3 cell lines. The doubling time of HeLaSI- and of Yoshida cells was increased by 12% and 10% respectively while the doubling time of HeLa CCL 2 did not change significantly. The metabolic responses of the two HeLa cell lines were +72–75% for lactate production and +54–68% for glucose consumption, while the corresponding figures for Yoshida Hepatoma cells were +24% and +19% only. The effect of MF-filtration on metabolism and growth could also be demonstrated if pieces of MF-filter were stirred in the complete medium for 30 min (not included in Table 1).

ing of the filters with 500 ml saline, which is routinely done, did not abolish the filter response.

The response could theoretically be caused by adsorption to the MF-filter of important growth- and metabolic factors; this was, up to a certain point, supported by the difference in response between HeLa cells and Yoshida cells, and also by the observation that no response was recorded when the filtration was carried out in the absence of calf serum, which was added later in the usual concentration. The hypothesis was, however, contradicted by an experiment where the filter-response in medium with 5% calf serum could not be compensated for by post-filtering supplementation with a further 5% calf serum, which would have supplied any serum component that might have been removed by the filter (Table 3).

It is more feasible, therefore, to believe that the

Table 1. Doubling time and metabolism of 3 cell lines cultured in MF-filtered medium (Millipore type MF-filter) and in non-filtered control medium.

Cells	Doubling time h			Lactate production pg cell^{-1} h^{-1}			Glucose consumption pg cell^{-1} h^{-1}		
	Control	Filtered	% Increase	Control	Filtered	% Increase	Control	Filtered	% Increase
HeLaSI	30·9	34·5	12	57·9	101·4	75	70·2	118·2	68
HeLaCCL2	21·6	21·7	·5	60·1	103·4	72	75·4	116·4	54
Yoshida hepatoma	16·7	18·4	10	18·7	23·2	24	25·3	30·2	19

Table 2 shows that filtration of serum-free culture medium though MF-filters did not induce alterations in growth and metabolism; the same was true when complete serum containing medium was filtered through the filters: 1120, OE66, BA83, and Durapore GVWP. Treatment of a MF-filter with 50% ethanol before filtration abolished its effect on growth and metabolism, whereas preliminary wash-

MF-filter supplies the media with a compound which is responsible for the rise in the metabolism and the inhibition of the growth. However, to explain the negative response to filtered serum-free media (Table 2) it must also be assumed that the compound is bound to the filter material in such a way that it can only be liberated in significant amounts if serum is present in the medium.

Table 2. Ratios of doubling time and metabolism of HeLaSI cells cultured in media filtered through 5 different filters.

% Calf serum during filtration	Remarks	Maker	Filter type	Ratios between experimental values and the corresponding control values: Doubling time	Lacate production	Glucose consumption
10		MP	MF	1·12	1·75	1·68
0	10% CS added after filtration	MP	MF	1·04	1·06	1·03
10	Alcohol treated filter	MP	MF	1·04	0·95	0·96
5		MP	Durapore (GVWP)	1·01	0·93	0·81
10		S&S	1120	0·96	1·03	1·10
5		S&S	OE66	1·01	0·97	1·09
5		S&S	BA83	1·10	1·11	0·97

MP = Millipore. S&S = Schleicher & Schüll. CS = Calf serum.

Table 3. Attempts to compensate for the MF-filter response of HeLaSI cells by supplementation with serum following filtration. Values related to 5% CS-control.

% Calf serum	Remarks	Doubling time	Lactate	Glucose
5	non-filtr. control	1·00	1·00	1·00
10	non-filtr. control	0·80	1·22	1·18
5	MF-filtered	1·23	1·88	1·97
5	MF-filtered and 5% CS added	1·02	1·98	1·92

Table 4 shows that dry matter from an ethanol extract on an MF-filter stirred into culture medium caused profound effects on metabolism and growth of HeLaSI cells cultured therein. The dry matter of ethanol extracts of the filters OE66, BA83, and Durapore GVWP did not significantly alter metabolism and growth of HeLaSI cells.

Fig. 1 demonstrates the absorption spectra of Triton-X-100 (A), and of an ethanol extract of an MF-filter. The points 1, 2, 3 and 4, 5, 7 correspond to identical wavelengths, namely 282, 276 and 226 nm. Point 6 corresponds to 252 nm. The main component of the extract is similar to Triton-X-100. The absorption at 252 nm may be due to presence of cellulose acetate in the extract, or that the two compounds are not completely identical, although they both belong to the Triton-X-series.

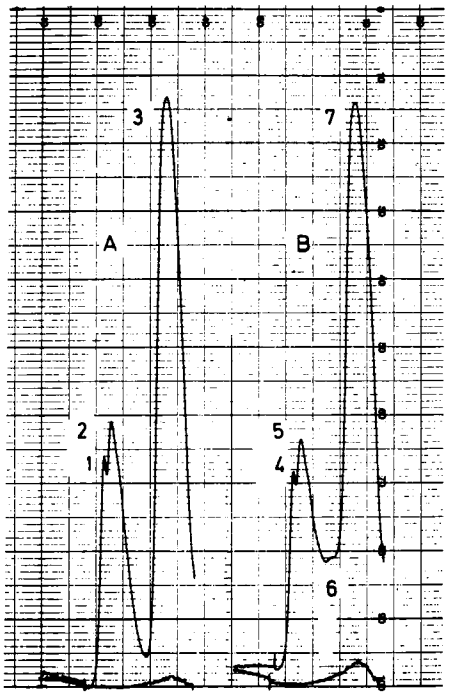

Fig. 1. Absorption spectra of Triton-X-100 (A), and of an ethanol extract of a MF-filter (B). The points 1, 2, 3 and 4, 5, 7 correspond to 282, 276 and 226 nm, respectively. Point 6 corresponds to 252 nm. Ordinate: Transmission %, Abscissa: Wavelengths nm.

Addition of 1–10 μg ml^{-1} of Triton X-100 to the culturing media gave straight line survival curves. Five μg ml^{-1} inhibited the growth of HeLaSI cells 40% and of Yoshida cells 20%. The lactate- and glucose metabolism of HeLaSI cells increases with the increasing concentration of Triton-X-100 (Fig. 2). Initially, the curve is steep, but flattens out and attains a maximum value at about twice the initial value. The response of Yoshida cells is much less, and the curves are not initially steep. Five μg ml^{-1} increased the metabolism of HeLaSI cells ca 100% and of Yoshida cells 30–50%.

Table 4. The effect on HeLaSI cells of treating culturing media with dry matter of ethanolic extract from 4 different filters. All media contained 10% calf serum.

Dry matter from: Maker	Type	Ratios between experimental values and the corresponding control values: Doubling time	Lactate production	Glucose consumption
MP	MF	2·64	1·91	1·78
MP	Durapore (GVWP)	·97	1·12	1·10
S&S	OE66	1·02	1·05	·95
S&S	BA83	·96	1·05	1·07

Triton-X-100-containing medium showed a striking similarity with filtered medium in its effect on HeLaSI- and Yoshida cells, both with respect to the relative modest effect on the growth and to the relatively large effect on the metabolism (Fig. 2). Further, the sensitivity of Yoshida cells was somewhat less than that of HeLaSI cells toward both media.

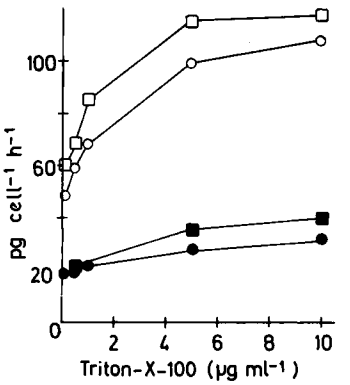

Fig. 2. Lactate production (○–○) or (●–●), and glucose consumption (□–□) or (■–■) of HeLaSI cells (upper two curves), and Yoshida cells (lower two curves) as a function of the Triton-X-100 concentration.

Reversibility of the filter response

HeLaSI cells could be maintained continuously on MF-filtered medium just as the control cells, but they did not grow up to quite the same density, probably because of their higher rate of lactate production.

HeLaSI cells reared on MF-filtered medium for up to 14 passages reduced their lactate and glucose metabolism and increased their growth rate as soon as they were grown in non-filtered medium. Thus MF-filtration did not seem to introduce irreversible alterations of HeLa cells even after prolonged cultivation in such media (up to 14 passages). No morphological changes or changes in the ploidy of the cells as judged by the DNA content, could be detected (Burton 1956; Leyva & Kelly 1974).

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