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LACK OF CORRELATION BETWEEN MEMBRANE LIPID COMPOSITION AND THERMOTOLERANCE IN CHINESE HAMSTER OVARY CELLS

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Membrane composition and fluidity, and survival of Chinese hamster ovary fibroblasts have been examined following various thermal exposures. It has been found that enhanced thermal resistance following brief exposure to 43°C is not accompanied by detectable membrane lipid alterations. This is in contrast to membrane alterations that occur following adaptation to elevated temperatures compatible with growth (39°C and 41°C).

The sensitivity of mammalian cells to elevated temperatures is dependent on the thermal history of the cell population, including both the growth temperature and previous exposures to thermal insult. Both exposure to non-lethal elevated temperatures, compatible with reproduction ($>37°C$ but $<41°C$) or brief exposure to lethal temperatures ($>41°C$) will result in subsequent enhanced resistance to lethal temperatures [1–3]. In general, the higher the temperature, the less exposure time required for development of an equivalent degree of subsequent thermotolerance [2].

It has been shown in a wide variety of organisms, both prokaryotic and eukaryotic, that the temperature of growth markedly affects cell membrane lipid composition (Ref. 1, see references cited). We recently examined Chinese hamster ovary fibroblasts in tissue culture grown for 72 h at 32°C, 37°C, 39°C, and 41°C (32°C and 41°C are the minimum and maximum temperatures at which growth was obtainable). We detected both the expected alterations in survival following a lethal thermal exposure (43°C) and pronounced alterations in membrane composition. In particular, in plateau phase cells increasing growth temperature correlated strongly with increase in the membrane cholesterol:phospholipid molar ratio and decrease in membrane fluidity as measured using fluorescence polarization [1]. This finding suggested that alterations in membrane composition may be responsible for acquired thermal resistance. In order to further examine this phenomenon, we determined to extend our investigation of membrane composition to the setting of thermotolerance arising after a brief temperature exposure in the lethal range (43°C). Despite the development of substantial thermotolerance, no significant alterations in membrane composition or fluidity were detectable.

All methods employed are as described previously [1]. Briefly, the Chinese hamster ovary fibroblast clone HA-1 (CHO HA-1) cell line was grown to early plateau phase prior to initiation of experiments. Cells were exposed to 43°C in specially constructed heat boxes [4]. Exposures to 37°C, 39°C, and 41°C were in incubators. At all times the atmosphere was regulated at 5% CO_2 and 95% air to maintain a constant pH of 7.4 ± 0.1.

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Survival assays were done using the cloning technique of Puck and Marcus [5]. Crude membrane fractions were prepared as described previously [1]. Briefly, whole cells were sonicated to ≥95% lysis and unbroken cells and nuclei sedimented on a clinical centrifuge. The supernatant was then centrifuged at 60000 × *g* for 30 min and the pellet taken as a crude membrane fraction. Protein content of the membrane fractions was assayed by the method of Lowry et al. [6], after a chloroform/methanol extraction of the membrane lipids. Bovine serum albumin was used as a standard. Total cholesterol in the lipid extract was determined by the method of Rouser et al. [8]. The acyl chain composition of the phospholipids was determined using gas-liquid chromatography as previously described [1]. Fluorescent polarization measurements employing diphenylhexatriene as the probe were done in a Spex fluorimeter at 23°C, as previously described in detail [1].

Table I shows cholesterol and phospholipid content and fluorescent polarization values for a crude membrane fraction isolated from Chinese hamster ovary cells. The cells were grown to plateau phase at 37°C and then exposed to the incubation conditions noted in the table prior to cell fractionation. In parallel experiments cells exposed to indentical conditions were assessed for survival following an exposure to 43°C for 120 min. As shown in experiment 1 when cells were grown at 39°C and 41°C there was an increase in membrane cholesterol:phospholipid ratio with a concomitant increase in survival following 43°C, 120-min exposure by factors of 27 and 47, respectively. The increasing fluorescence polarization, indicating decreased probe rotational mobility, as measured at 23°C, reflects the increasing membrane cholesterol relative to phospholipid due to thermal adaptation. In experiment 2 an even larger enhancement of cellular survival (factor of 80) was obtained 6 h after a brief exposure to 43°C. However, no significant changes in cholesterol:phospholipid molar ratio or polarization were detected.

Previously, fatty acid chain composition of membrane phospholipids was evaluated in experiments comparable to those illustrated in experiment I of Table I [1]. In those experiments no significant differences in acyl chain composition between the experimental groups were detected. Similar analyses were completed following the conditions in experiment 2. As shown in Table II no significant differences between the groups were detected. We point out, however, that in both groups there was a fraction of unidentified, long chain phospholipids (approx. 20% of total) and that changes within that fraction would not have been detected in our experiments.

Under the conditions we have studied, following the initial heat shock at 43°C maximal acquired thermal resistance is seen by 6 h, at which time we evaluated membrane composition. We were unable to detect alteration in membrane lipid composition

TABLE I

Expt. No.	Incubation conditions	Cholesterol/mg protein (μg)	Phospholipid/mg protein (μg)	Cholesterol: phospholipid molar ratio	Polarization	Surviving fraction following exposure to 43°C for 120 min
1	37°C × 72 h	151 ± 3(4) [a]	484 ± 9(4)	0.59 ± 0.01(4)	0.223 ± 0.001(5)	$1.5 \cdot 10^{-2}$ [b]
	39°C × 72 h	202 ± 11(4)	551 ± 16(4)	0.69 ± 0.02(4)	0.237 ± 0.001(6)	$4 \cdot 10^{-1}$
	41°C × 72 h	190 ± 4(4)	428 ± 8(4)	0.84 ± 0.03(4)	0.243 ± 0.001(6)	$7 \cdot 10^{1}$
2	37°C × 6 h	132 ± 2(4)	396 ± 13(4)	0.63 ± 0.02(4)	0.282 ± 0.001(8)	$1 \cdot 10^{2}$
	43°C × 30 min followed by 37°C × 6 h	146 ± 5(4)	426 ± 9(4)	0.65 ± 0.02(4)	0.280 ± 0.002(8)	$8 \cdot 10^{-1}$

[a] Standard error based on number of determinations indicated in parentheses.

[b] Standard deviations of survival determinations are less than 25% of survival values.

TABLE II

FATTY ACYL CHAIN COMPOSITION OF PHOSPHOLIPIDS

Each result is the average of two experiments, expressed as percent of total fatty acids.

Incubation conditions	Fatty acid type									Unidentified
	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:4	
37°C×6 h	3.5	2.1	13.5	11.3	10.0	21.2	9.1	4.8	6.9	18.4
43°C×30 min, followed by 37°C×6 h	2.8	2.4	12.1	11.6	9.0	20.7	9.6	4.2	6.3	21.5

or fluidity in CHO cells at that time. Similar results in Ehrlich ascites cells showing no change in membrane parameters during development of thermotolerance following brief exposure to lethal temperature (43°C) have been obtained by Anderson and Parker [9].

Li et al. [2] have found that enhancement of thermal resistance arises during continuous incubation at 41°C plateaus of about 8 h. We have also evaluated membrane composition and fluidity at eight hours during continuous exposure to 41°C. We found that no significant changes were detectable in either lipid composition or fluorescence polarization (data not shown). It therefore appears that membrane composition alterations seen during prolonged incubation at 41°C occur sometime between 8 and 72 h, later than the onset of cellular heat resistance.

Based on earlier work [1], we had suggested the hypothesis that cellular heat resistance was mediated by alterations in membrane lipid composition, in particular the cholesterol:phospholipid ratio. The present findings indicate that acquisition of thermotolerance may result from mechanisms not related to changes in membrane lipid composition in Chinese hamster ovary fibroblasts.

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