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MANIPULATION OF FATTY ACID COMPOSITION OF MEMBRANE PHOSPHOLIPID AND ITS EFFECTS ON CELL GROWTH IN MOUSE LM CELLS

OSAMU DOI [a,b,*], FUMIKO DOI [a,b], FRIEDHELM SCHROEDER [a,**], ALFRED W. ALBERTS [a,b] and P. ROY VAGELOS [a,b]

[a] *Department of Biological Chemistry, Washington University, St. Louis, Mo. 63110 and* [b] *Merck Sharp and Dohme Research Laboratories, Rahway, N.J. 07065 (U.S.A.)*

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Summary

Fatty acid composition of the phospholipids of mouse LM cells grown in suspension culture in serum-free chemically defined medium was modified by supplementing the medium with various fatty acids bound to bovine serum albumin.

Following supplementation with saturated fatty acids of longer than 15 carbons (100 μM) profound inhibition of cell growth occurred; this inhibitory effect was completely abolished when unsaturated fatty acids were added at the same concentration. Supplementing with unsaturated fatty acids such as linoleic acid, linolenic acid or arachidonic acid had no effect on the cell growth.

Fatty acid composition of membrane phospholipids could be manipulated by addition of different fatty acids. The normal percentage of unsaturated fatty acids in LM cell membrane phospholipids (63%) was reduced to 35—41% following incorporation of saturated fatty acids longer than 15 carbon atoms and increased to 72—82% after addition of unsaturated fatty acids.

A good correlation was found between the unsaturated fatty acid content of membrane phospholipids and cell growth. When incorporated saturated fatty acids reduced the percentage of unsaturated fatty acids in membrane phospholipids to less than 50%, severe inhibition of the cell growth was found. Simultaneous addition of an unsaturated fatty acid completely abolished this effect of saturated fatty acids.

* Present address: Department of Chemistry, National Institute of Health, 2-10-35 Kamiosaki, Shinagawa-Ku, Tokyo, 141, Japan.

** Present address: Department of Pharmacology, Medical School, University of Missouri, Columbia, Mo. 65201, U.S.A.

Abbreviations: lauric acid (12 : 0), tridecanoic acid (13 : 0), myristic acid (14 : 0), pentadecanoic acid (15 : 0), palmitic acid (16 : 0), heptadecanoic acid (17 : 0), stearic acid (18 : 0), nonadecanoic acid (19 : 0), arachidic acid (20 : 0), myristoleic acid (14 : $1\Delta^{9}$), 10-pentadecenoic acid (15 : $1\Delta^{10}$), palmitoleic acid (16 : $1\Delta^{9}$), 10-heptadecenoic acid (17 : $1\Delta^{10}$), oleic acid (18 : $1\Delta^{9}$), 10-nonadecenoic acid (19 : $1\Delta^{10}$), 11-eicosanoic acid (20 : $1\Delta^{11}$), linoleic acid (18 : $2\Delta^{9,12}$), linoleic acid (18 : $3\Delta^{9,12,15}$), 8,11,14-eicosatrienoic acid (20 : $3\Delta^{8,11,14}$), and arachidonic acid (20 : $4\Delta^{5,8,11,14}$).

The results suggest that maintenance of membrane fluidity by unsaturated fatty acids in membrane phospholipids is critical to membrane integrity and cell growth.

Introduction

The major components of biological membranes include proteins, sterols and phospholipids. All of these are critical to the maintenance of membrane integrity and in regulation of various membrane functions. One approach to the study of the role of phospholipids in membrane structure and function has been to observe effects associated with modification of the polar head groups and fatty acid compositions of membrane phospholipids [1—12]. Alteration of polar head group composition by substitution of choline with its analogues has been accomplished in *Neurospora crassa* mutants [1,2], LM cells [3—11], and whole animals [12]. These studies demonstrated that changes in polar head group composition of LM cells could alter some membrane enzyme characteristics [9] and caused small, but critical, differences in membrane content of long-chain fatty acids, sphingomyelin, lysophosphatidylcholine and ether-bonded lipids [3,6,7]. Alteration of acyl groups of phospholipids in microorganisms [13,14] caused significant changes in the physical properties of the biological membranes. Although changes in phospholipid fatty acid composition have also been induced in mammalian cells, only minor effects on cellular metabolism have been observed [3,15—19].

The results reported here extend previous observations concerning the effects on cell growth and fatty acid composition of membrane phospholipids in LM cells which occur when exogenous fatty acids are supplied in the growth medium.

Our results demonstrate that cell growth ceases when more than 50% of the acyl groups of phospholipids are saturated fatty acids. The effects of these manipulations of the acyl groups of membrane phospholipids on cellular lipid metabolism and similar studies following modification of phospholipid polar head groups in these cells will be reported elsewhere (Doi, O., Doi, F., Schroeder, F., Alberts, A.W. and Vagelos, P.R., unpublished).

Materials and Methods

All radioactive chemicals were obtained from New England Nuclear Corp. Fatty acids were obtained from Nu Check Prep and Sigma. Mouse LM cells (American Type Culture Collection) were grown in suspension culture in serum-free, modified Higuchi medium as previously described [6]. The cells were cultured in suspension in 100-ml serum bottles containing 10—40 ml of medium on a New Brunswick Gyrotory Shaker at 140 rev./min. In addition to the modifications of the Higuchi medium previously described [6], the medium contained 25 μM fatty acid-free bovine serum albumin (Pentex, Miles Laboratories). In order to eliminate any effect of bovine serum albumin on cellular metabolism, cells were grown in the presence of fatty acid-free bovine serum albumin for at least 2 weeks prior to use. Under these conditions the cell

density increased 6–8-fold in 6 days when the initial cell density was $5 \cdot 10^5$ cells/ml. Greater than 90% of the cells were viable as determined by dye exclusion with Trypan Blue.

Unlabeled or 1-^{14}C-labeled fatty acids were bound to fatty acid-free bovine serum albumin by the method of Spector and Hoak [20] and fatty acid content of the complex was determined by the titration method of Dole [21]. Initially, fatty acid · bovine serum albumin complexes contained either 6–10 μmol of fatty acids per μmol of bovine serum albumin or $1.6 \cdot 10^7$ cpm of fatty acid per μmol of bovine serum albumin and were stored at −20° C after sterilization through a 0.22 μm Millipore filter. These stock solutions of fatty acid · bovine serum albumin complexes were mixed with fatty acid-free bovine serum albumin to give the final concentrations of fatty acid and total bovine serum albumin given in legends to tables and figures. Purity of fatty acids in fatty acid · bovine serum albumin complexes was determined by analytical and/or preparative gas-liquid chromatography after methylation of the fatty acids [3,6]. In all cases, purity of the fatty acids in the complexes was greater than 98% and the radiochemical purity was greater than 97%. Following incubation of LM cells with various added fatty acids (specifics given in tables and figures), the cells were harvested and phospholipids extracted and separated from neutral lipids as previously described [3,6]. Analyses of phospholipid fatty acids were performed following preparation of fatty acid methyl esters as previously described [3,6]. The samples were dissolved in carbon disulfide and analyzed by gas-liquid chromatography at 170° C on a 6 ft column of 10% DEGS on gas chrom W, 60–80 mesh with a Varian Model 2100 gas chromatograph. For the radioactive gas-liquid chromatography analysis, a Varian Model 2100 gas chromatograph equipped with 10 : 1 glass splitter was used. After each injection, each fraction was collected with solid CO_2 cooled glass tubes (0.3 m × 3 mm) and eluted with 10 ml of Aquasol scintillation fluid (New England Nuclear). Fatty acid methyl esters were identified by comparison of their retention times with those of authentic standards (Nu Check Prep and Sigma). The peak areas were measured using Varian CDS-101 chromatography data system.

Results

Effects of exogenous fatty acids on growth of LM cells

Fig. 1 demonstrates the effects of the addition of different individual fatty acids complexed to bovine serum albumin on the growth rate of LM cells in suspension culture. Lauric acid (12 : 0) and all of the mono- and polyunsaturated fatty acids tested did not affect LM cell growth when added at a concentration of 100 μM. Tridecanoic acid (13 : 0) and myristic acid (14 : 0) moderately inhibited cell growth while saturated fatty acids with longer chain lengths (15 : 0–20 : 0) caused a marked reduction in cell number.

The effect of palmitic acid (16 : 0) on cell number was concentration dependent (Fig. 2). At higher concentrations (100 or 200 μM), cell number decreased markedly with 2 days. However, at 50 μM palmitic acid (16 : 0) cell number decreased only slightly for the first 3–4 days and then began to increase rapidly. There was no difference in sensitivity to 50 μM palmitic acid among

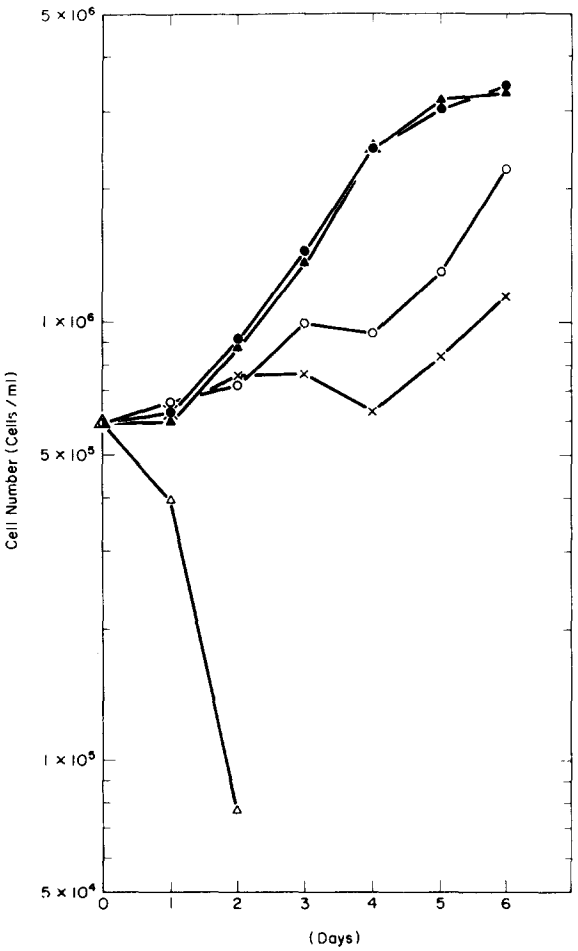

Fig. 1. Effect of individual fatty acid supplements on cell growth. LM cells were suspended in individual bottles of medium containing one of the following fatty acids complexed to fatty acid-free bovine serum albumin (final concentration of fatty acids was 100 μM and bovine serum albumin was 25 μM): no added fatty acid or added 12 : 0 (●———●, identical results shown as a single curve), 13 : 0 (○———○); 14 : 0 (X———X); 15 : 0, 16 : 0, 17 : 0, 18 : 0, 19 : 0 or 20 : 0 (△———△, identical results expressed as a single curve); $14:1\Delta^{9}$, $15:1\Delta^{10}$, $16:1\Delta^{9}$, $17:1\Delta^{10}$, $18:1\Delta^{9}$, $19:1\Delta^{10}$, $20:1\Delta^{11}$, $18:2\Delta^{9,12}$, $18:3\Delta^{9,12,15}$ or $20:4\Delta^{5,8,11,14}$ (▲———▲), identical results expressed as a single curve. Cells were cultured in suspension at 37°C with rotary shaking. The initial cultures contained 15 ml of medium with $5 \cdot 10^5$ cells/ml. 1 ml of fresh medium (identical in fatty acid composition to the initial medium of that bottle) was added daily to avoid deposition of cells on the bottle walls near the upper surface of the medium. Cell number was determined daily on a small aliquot and corrected for the change in culture volume.

cells previously grown with no fatty acid supplement and those previously grown in the presence of 50 μM palmitic acid (Fig. 2). This result suggested that the LM cells (at this cell density) could metabolize added palmitic acid (at 50 μM) at a rate rapid enough to allow survival and reduce the concentration of 16 : 0 sufficiently to then allow normal growth.

The lethal effect of 100 μM palmitic acid was completely prevented by simultaneous addition of 100 μM unsaturated fatty acids (Fig. 2).

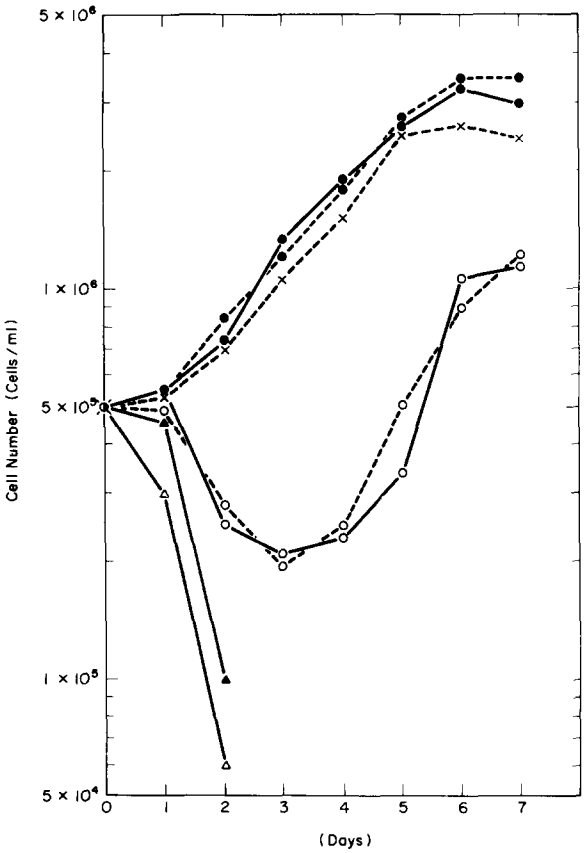

Fig. 2. Effects of supplementation with various concentrations of saturated and unsaturated fatty acids on the cell growth. LM cells previously cultured in fatty acid-free bovine serum albumin (25 μM) were harvested and resuspended in medium without added fatty acid (●———●); with 16 : 0 at 50 μM (○———○), 100 μM (▲———▲) or 200 μM (△———△); with 16 : 0 and 16 : 1Δ^9 at 100 μM each (●- - - - -●); or with 16 : 0 and 18 : 1Δ^9 at 100 μM each (X- - - - -X). Cells previously grown in the presence of 50 μM 16 : 0 for 7 days were suspended in medium containing 50 μM of 16 : 0 (○- - - - -○). Other culture conditions and analysis were as described in Materials and Methods and legend to Fig. 1.

Changes in fatty acid composition of phospholipids following supplementation with various fatty acids

Following addition of individual fatty acids (100 μM) bound to bovine serum albumin to LM cell cultures for a period of 24 h, the cells were harvested and the content of various fatty acids in total phospholipids was analyzed (Tables I and II). Differences in cell density of cultures supplemented with various fatty acids for this length of time were minimal (Figs. 1 and 2). Shorter chain fatty acids (12 : 0, 13 : 0, 14 : 0) were not extensively incorporated into LM cell phospholipids, although supplementation with these fatty acids did alter cellular lipid metabolism (Doi, O. et al., unpublished). All other fatty acids tested were effectively incorporated into cellular phospholipids, resulting in major modifications of their fatty acid content.

Addition of each long chain saturated fatty acid (15 : 0—20 : 0) caused a marked increase in the amount of that fatty acid found in phospholipids

TABLE I

FATTY ACID COMPOSITION OF PHOSPHOLIPIDS OF LM CELLS GROWN IN THE PRESENCE OF FATTY ACIDS

Cells were supplemented with 100 μM of fatty acid bound to bovine serum albumin (25 μM), for 24 h, harvested and the lipids analyzed as previously described [3, 6].

(A) Supplementation with saturated fatty acids

Supplemented fatty acid	Fatty acid content (percent by weight) **													Unsaturated fatty acids (%)
	14 : 0	15 : 0	16 : 0	16 : 1	17 : 0	17 : 1	18 : 0	18 : 1	19 : 0	19 : 1	20 : 0	20 : 1	20 : 2—20 : 4	
None	— *	—	15.0	5.4	—	1.0	12.5	52.7	—	—	—	3.7	6.0	68.8
12 : 0	2.7	—	18.0	5.7	—	1.0	13.9	47.0	—	—	—	3.1	6.2	63.0
13 : 0	—	7.3	16.9	4.9	3.0	2.0	14.0	40.8	—	—	—	2.5	5.0	55.7
14 : 0	*14.5*	—	19.2	7.4	—	—	12.0	36.2	—	—	—	3.1	5.1	51.8
15 : 0	—	*33.5*	9.6	2.5	7.2	4.4	9.3	23.5	—	—	—	1.6	6.0	38.0 †
16 : 0	1.1	—	*44.7*	8.3	—	1.0	10.5	24.6	—	—	—	2.0	5.1	41.0 †
17 : 0	—	2.3	7.2	1.3	*43.9*	10.0	7.6	20.6	—	—	—	1.4	3.6	36.9 †
18 : 0	—	—	10.0	3.7	—	—	*49.4*	26.2	—	—	—	1.5	4.9	36.3 †
19 : 0	—	1.8	9.4	1.1	7.3	1.5	7.5	23.4	*35.5*	6.1	—	1.4	3.7	37.2 †
20 : 0	—	—	12.0	2.9	—	—	16.5	29.5	—	—	*32.3*	—	2.8	35.2 †

(B) Supplementation with unsaturated fatty acids.

Supplemented fatty acid	Fatty acid content (percent by weight) ***											Unsaturated fatty acids (%)
	14 : 1	15 : 1	16 : 0	16 : 1	17 : 1	18 : 0	18 ; 1	18 : 2	18 : 3	19 : 1	20 : 1	20 : 2—20 : 4	
14 : 1Δ9	*3.3*	—	15.4	14.1	1.0	12.5	42.0	—	—	—	2.2	5.4	68.0
15 : 1Δ10	—	*32.7*	10.2	2.7	7.5	10.1	27.0	—	—	—	2.7	4.3	76.9
16 : 1Δ9	—	—	14.0	*30.3*	—	11.9	35.6	—	—	—	3.0	2.2	71.1
17 : 1Δ10	—	—	12.1	3.5	*35.2*	10.0	31.6	—	—	—	2.9	2.8	76.0
18 : 1Δ9	—	—	10.7	4.5	—	9.8	*67.2*	—	—	—	2.7	2.7	77.1
19 : 1Δ10	—	—	9.3	4.0	—	7.5	38.7	—	—	*33.7*	2.6	3.0	82.0
20 : 1Δ11	—	—	8.5	4.1	—	6.5	39.5	—	—	—	*34.4*	3.5	81.5
18 : 2Δ9,12	—	—	11.1	3.0	—	7.8	15.1	*46.2*	—	—	3.0	11.1	78.4
18 : 3Δ9,12,15	—	—	13.0	3.5	-	19.9	23.2	—	*34.3*	—	—	3.5	64.5
20 : 4Δ5,8,11,14	—	—	20.5	4.2	—	18.8	18.7	—	—	—	3.0	*37.2*	60.1

* Indicates fatty acid content of less than 1%.

** The percentages of 12 : 0, 13 : 0, 14 : 1, 15 : 1, 18 : 2 and 18 : 3 were less than 1% in all cases.

*** The percentages of 14 : 0, 15 : 0, 17 : 0, 19 : 0 and 20 : 0 were less than 1%.

† Note that supplementation with these fatty acids (compare with Fig. 1) resulted in a marked reduction of cell number when continued for 2 days.

TABLE II

CHANGE OF THE FATTY ACID COMPOSITION AND THE PERCENTAGE OF UNSATURATED FATTY ACID IN PHOSPHOLIPID WITH THE MIXED SUPPLEMENT OF FATTY ACIDS

Cells were cultured in the presence of various fatty acids for 24 h as described in Materials and Methods and legend to Fig. 2. Cells were harvested and cellular lipid analyzed as previously described [3,6].

Supplemented fatty acids	Fatty acid composition *				Unsaturated fatty acid (%) **
	16 : 0	16 : 1	18 : 0	18 : 1	
None	15.0	6.6	11.5	47.3	64.1
16 : 0					
50 μM	*36.1*	7.1	9.2	33.1	47.8
100 μM	*48.5*	6.4	7.3	26.9	39.4 ***
200 μM	*60.5*	3.3	5.2	18.0	28.0 ***
16 : $1\Delta^9$ (100 μM)	11.2	*29.5*	8.3	37.7	74.6
18 : $1\Delta^9$ (100 μM)	8.8	4.9	8.5	*64.8*	78.5
16 : 0 (100 μM) + 16 : $1\Delta^9$ (100 μM)	*30.7*	*21.5*	8.3	28.6	56.2
16 : 0 (100 μM) + 18 : $1\Delta^9$ (100 μM)	*25.7*	5.0	6.7	*51.5*	62.4
Fatty acid mixture (200 μM)	16.5	7.0	12.5	49.0	65.2

* Percent in total fatty acid in phospholipid by weight.

** Percent of unsaturated fatty acid in total fatty acid of phospholipid by weight.

*** Mixture of 16 : 0 (20%), 16 : $1\Delta^9$ (10%), 18 : 0 (15%) and 18 : $1\Delta^9$ (55%).

(Table IA, italic values) with a concomitant decrease in the percent of unsaturated fatty acids (Table IA, final column). Reduction in total unsaturated fatty acids (Table IA, final column) paralleled the reduction in cell number associated with addition of these long chain saturated fatty acids (compare with growth curves shown in Fig. 1).

Addition of individual unsaturated fatty acids for 24 h resulted in marked increases of these fatty acids in membrane phospholipids (Table IB, italic values). Although mild decreases in saturated fatty acid content occurred, simultaneous decreases in oleic acid (18 : $1\Delta^9$) minimized increases in the total percent of unsaturated fatty acids (Table IB, final column).

Supplementation of LM cell cultures with various concentrations of palmitic acid (16 : 0) or of mixtures of 16 : 0 and unsaturated fatty acids also allowed efficient incorporation of both species into membrane phospholipids (Table II, italic values). Addition of 16 : 0 alone resulted in a marked reduction of toal unsaturated fatty acids, especially at the higher concentrations.

When mixtures of palmitic acid (16 : 0) and either 16 : $1\Delta^9$ or 18 : $1\Delta^9$ were added to cultures, incorporation of each fatty acid into phospholipids (as compared to addition of either alone) was reduced. This reduced, but balanced incorporation of both species allowed total unsatured fatty acid contents to remain above 56%. Similar results were obtained when mixtures of either heptadecanoic acid (17 . 0), stearic acid (18 : 0) or nonadecanoic acid (19 : 0) with linoleic acid (18 : $2\Delta^{9,12}$) were added (data not shown).

In all of the experiments shown in Tables I and II, the presence of more than 56% total unsaturated fatty acids in membrane phospholipids was associated with continued logarithmic cell growth (compare with Figs. 1 and 2). Total unsaturated fatty acid contents of less than 41% were associated with drastic

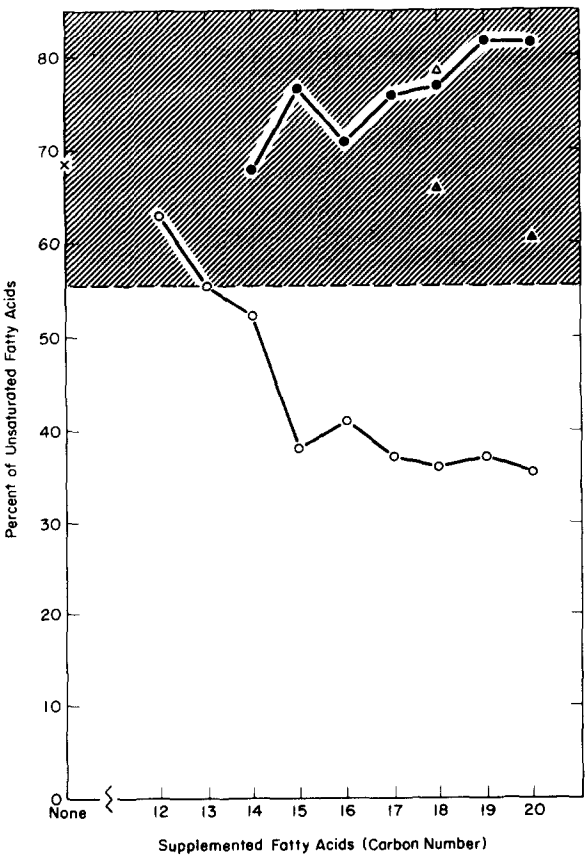

Fig. 3. Percent of unsaturated fatty acids in phospholipids following supplementation with various fatty acids. The data shown in the final column of Tables IA and IB are plotted to emphasize the changes in total phospholipid unsaturated fatty acid content. Values in shaded area represent the fatty acid composition of cells with demonstrated continued logarithmic growth (derived from data in Figs. 1 and 2). ○———○, added saturated fatty acids; ●———●, mono-saturated fatty acids; △, $18:2\Delta^{9,12}$; ▲, $18:3\Delta^{9,12,15}$; ▲, $20:4\Delta^{5,8,11,14}$.

reductions in cell number. Unsaturated fatty acids contents of intermediate percentages (42—55%) (as with 13 : 0, 14 : 0 and 50 μM 16 : 0) were associated with a slowed rate of cell growth. The results also showed that moderate reductions in the amount of saturated fatty acids did not adversely affect cell growth. These conclusions are graphically summarized in Fig. 3.

Mechanism of incorporation of mixtures of fatty acid supplements

In order to show that balanced incorporation of mixtures of saturated and unsaturated fatty acid supplements was not due to selective incorporation of one species, cells were incubated with various mixtures of saturated and unsaturated fatty acids (Table II). The results show that incorporation of 16 : 0 into phospholipids was reduced to the same extent by equimolar amounts of either $16:1\Delta^9$ or $18:1\Delta^9$. Moreover, when a mixture of four fatty acids (Table II) in the proportions found in LM cell phospholipids was supplemented, fatty acid composition was unchanged from that found in unsupple-

TABLE III

METABOLISM OF INCORPORATED LABELED FATTY ACIDS

Cells (10^6 cells/ml) were grown in the presence of 100 μM 1-^{14}C-labeled fatty acid (1.2—1.4 Ci/mol) for 12 h. Labeled fatty acids in phospholipid were analyzed by the preparative gas-liquid chromatography (see Materials and Methods). About one-third of the supplemented fatty acids was incorporated within 12 h.

Supplemented fatty acid	Distribution of fatty acids (percent of radioactivity)										
	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3	20 : 0	20 : 1	20 : 2	20 : 3	20 : 4
[1-^{14}C]16 : 0	64.3	8.7	17.4	6.2	—*	—	—	—	—	—	—
[1-^{14}C]18 : 0			65.7	29.4	—	—	1.3	—	—	—	—
[1-^{14}C]18 : 2$\Delta^{9,12}$					88.8	—	—	—	7.8	1.0	—
[1-^{14}C]20 : 3$\Delta^{8,11,14}$										63.1	37.0

* Indicates less than 1%.

mented cells. These data suggested that, following addition of mixtures of fatty acids, selective incorporation of any one species did not occur.

Further metabolism of added fatty acids

The results shown in Table I and II demonstrated that the added short chain fatty acids could be elongated and then incorporated into membrane phospholipids. Lauric acid (12 : 0) was readily converted to 14 : 0, 13 : 0, to 15 : 0 and 17 : 0; 14 : $1\Delta^9$, to 16 : 1; and 15 : $1\Delta^{10}$, to 17 : 1. However, elongation of added longer chain fatty acids (17 : 0, 18 : 0, 17 : $1\Delta^{10}$, 18 : $1\Delta^9$ and 18 : $2\Delta^{9,12}$) was poor.

An increase in the amount of desaturated products derived from supplemented fatty acids was also detected (from 16 : 0 to 16 : 1; from 17 : 0 to 17 : 1; and from 19 : 0 to 19 : 1). However, in none of these cases was the amount of desaturation sufficient to compensate for the associated decrease in total unsaturated fatty acids found.

Some evidence for β-oxidation of added 17 : 0 or 19 : 0 was also observed.

The metabolic fate of the supplemented fatty acids was further studied using incorporation of 1-^{14}C-labeled fatty acids and analysis of the distribution of radioactivity in fatty acids of membrane phopholipids (Table III). The position of label in fatty acid eliminated the possibility that the measurement of radioactivity would reflect any β-oxidation of these incorporated fatty acids. [1-^{14}C]Palmitic acid (16 : 0) was readily desaturated (to 16 : $1\Delta^7$ and 18 : $1\Delta^9$) and elongated (to 18 : 0). Although [1-^{14}C]stearic acid (18 : 0) was also desaturated well, only minimal elongation of 18 : 0 occurred. Elongation of incorporated [1-^{14}C]linoleic acid (18 : $2\Delta^{9,12}$) occurred while desaturation was minimal. On the other hand, 1-^{14}C-labeled 20 : $3\Delta^{8,11,14}$ was extensively desaturated to 20 : 4. These results concerning metabolism of incorporated linoleic acid and 8,11,14-eicosatrienoic acid were consistent with a previous report [22].

Discussion

We have used incorporation of exogenous acids into LM cells in suspension culture to study the relationship between cell growth and changes in fatty acid composition of membrane phospholipids. Our results (compare Figs. 1, 2 and 3) demonstrated that only if more than 56% of the fatty acids of membrane phospholipids were unsaturated was normal cell growth maintained. Increases in the percentages of unsaturated fatty acids from the normal 65% to 80% did not affect growth rate [23—25]. Reductions of unsaturated fatty acid content to the range or 48—55% (as with addition of 13 : 0, 14 : 0 and 50 μM 16 : 0) were associated with decreases in gorwth rate. In these experiments, incorporation of added shorter chain saturated fatty acids (13 : 0 and 14 : 0, for example) did not extensively modify fatty acid composition of phospholipids. Conceivably, if these short chain fatty acids had been added at higher concentrations, more extensive modifications of fatty acid content might have been associated with more severe reductions in growth rate (as demonstrated for 16 : 0). More severe decreases in the percentage of unsaturated fatty acids (to 30—45%) associated with incorporation of saturated fatty acids were followed

by marked reductions in cell number. The effects of saturated fatty acid 16 : 0) supplementation were dependent on both the concentration of fatty acid added and cell density. The toxic effects of saturated fatty acid (16 : 0) supplementation could be prevented by addition of equimolar amounts of any of several unsaturated fatty acids, which resulted in maintenance of the unsaturated fatty acid content above 56%. On the other hand, when the percentage of saturated fatty acids was reduced (from the normal of 31% to 18%) no deleterious effect on cell growth was found. These results suggest that an adequate percentage of unsaturated fatty acids in membrane phospholipids is necessary to maintain membrane integrity. Previous studies [13,14] with prokaryotic and artificial membranes have demonstrated that changes in saturated fatty acid content resulted in marked changes in membrane fluidity. Simple calculations of total percent of unsaturated fatty acids (as shown in Fig. 3) do not take into account either chain lengths of fatty acids or degrees of unsaturation of fatty acids, both of which are known to affect measurements of membrane fluidity. Nonetheless, the correlation of unsaturated fatty acid content with changes in cell growth rates which we have demonstrated clearly suggests that changes in membrane fluidity may adversely affect cell growth.

Our studies also allow some conclusions concerning the ability of LM cells to elongate and desaturate incorporated fatty acids (Tables I and III). In our experiments, significant elongation of all fatty acids of chain lengths less than 17 and of 18 : $2\Delta^{9,12}$ occurred. Elongation of the other fatty acids was not observed. These results demonstrate the specificity of the elongation reaction in LM cells for saturated and mono-unsaturated fatty acids with chain lengths of less than 17 carbons. Significantly, there was no desaturation of 18 : $3\Delta^{9,12,15}$ which is the first step in the formation of 4,7,10,13,16,19-docosahexoaenoic acid from linoleic acid. Desaturation of incorporated 16 : 0, 17 : 0, 18 : 0, 19 : 0 and 20 : $3\Delta^{8,11,14}$ was detected but no desaturation of 15 : 0, 20 : 0 or 18 : $2\Delta^{9,12}$ was found. These results are consistent with several previous reports [17,22]. However, it is noteworthy that when large amounts of saturated fatty acids were incorporated (as with supplemented 15 : 0—20 : 0), the amount of desaturation was not sufficient to maintain the high levels of unsaturated fatty acids in membrane phospholipids associated with continued normal cell growth.

Several recent studies have also demonstrated changes in mammalian cell membrane functions and structure following alteration of fatty acid content of membrane phospholipids. Engelhard et al. [9] showed that changes in plasma membrane fatty acids affected the activity, hormone response and temperature dependence of adenylate cyclase. Chang and Vagelos [26] during isolation and characterization of a mutant Chinese hamster ovary cells deficient in desaturase activity demonstrated that growth of both wild type and mutant cells was inhibited by palmitic acid. Moreover, viability of the mutant cells was totally dependent upon addition of exogenous unsaturated fatty acids. These and other previous studies have shown deleterious effcts upon cells under conditions of unsaturated fatty acid deficiency. However, the quantitative analysis of membrane phospholipid fatty acid composition provided in this report and the correlation of percentage of unsaturated fatty acid content with cell growth give a clearer insight into one possible mechanism of the deleterious effects.

Clearly, manipulation of fatty acid content in LM cell membranes, in conjunction with alteration of polar head group composition, will continue to be useful in understanding the role of these components in the structure and function of the membranes.

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