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UTILIZATION OF EXOGENOUS LINOLENIC AND OLEIC ACIDS FOR PLASMA MEMBRANE PHOSPHOGLYCERIDE SYNTHESIS IN L-FIBROBLASTS

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Summary

The inability of the strain L-fibroblast to synthesize quantitatively significant amounts of polyenoic fatty acid and the apparent lack of turnover of their phosphoglyceride acyl groups under the usual conditions of cell culture makes them especially well suited for studies concerning the effect of fatty acid unsaturation on biological membranes. Such cells grown in the absence of exogenous lipid sources have in their phosphoglycerides only traces of polyenoic fatty acid.

By infusing fatty acid supplements into suspension cultures of logarithmically growing cultures of L-fibroblasts it is possible to increase significantly their phosphoglyceride polyenoic fatty acid content to as much as 50% of the total lipid phosphoglyceride fatty acids. The infusion of fatty acid supplements at a constant rate over a 48 h time period diminishes the toxic effects which may accompany single doses of unesterified fatty acids and reduces considerably the accumulation of cytoplasmic lipid droplets. Cultures supplemented in this way have virtually the same generation times as non-supplemented control cultures. The data show that alterations in surface membrane and homogenate polyenoic fatty acid composition are minimal when oleic acid is supplied to the culture. During exposure to large amounts of polyenoic fatty acid, however, the unsaturation of plasma membrane total phosphoglyceride fraction is less than that of the cell homogenate. This effect is more pronounced in the phosphatidylethanolamine than in the phosphatidylcholine fraction.

Introduction

Through work with bacterial auxotrophs, it has been shown that the physical-chemical properties of membranes may be affected by alterations in their

phosphoglyceride polyenoic fatty acid content [1—3]. More recently observations of the effect of alterations in membrane lipid acyl group composition on membrane properties have been extended to mammalian cell culture systems [4—8]. In some such studies, fatty acids were added to cultured fibroblasts either as albumin complexes or as Tween esters. This resulted in an increase in phosphoglyceride polyenoic fatty acid content to as much as one-third of the total phosphoglyceride fatty acids. In at least one instance a marked loss of cell viability occurred during the incubation [8].

Since compositional changes occurring in the mammalian cell phosphoglyceride fatty acids result in altered transport phenomena similar to that described in bacterial membranes [8], it is important to know whether the fatty acid composition of the mammalian cell outer membrane is regulated in any manner. A recent study [5] has shown that after a modest increase from a baseline value of 8% up to 23% in the linoleic acid content of the outer membrane of Ehrlich ascites tumor cells, the fatty acid composition of the outer membrane was accurately represented by the fatty acid composition of the total cell phospholipid. The objective of the present study was to produce a more marked increase in the polyenoic fatty acid content of mammalian cell phosphoglycerides, without associated toxicity, and to determine whether in such cells there is evidence for compensating mechanisms which regulate the phosphoglyceride fatty acid composition of the outer membrane.

Methods

Tissue culture

Littlefield strain L-fibroblasts were grown in 50 ml Waymouth's MB 752/1 medium (Associated Biomedic Systems, Inc., Buffalo, N.Y.) and supplemented with 2.5% delipidized horse serum [9] as described previously [10]. Cell numbers were monitored with a Model B Coulter Counter (Coulter Electronics, Hialeah, Fla.) and were maintained in the log phase of growth, between $5.0 \cdot 10^5$ to $1.2 \cdot 10^6$ cells per ml, by dilution with fresh medium. Cells grown in this manner maintained a doubling time of approximately 24 h.

Fatty acid supplementation

Fatty acids were obtained from Supelco (Bellefonte, Pa.), dispensed under N_2 at 4°C into heptane (10 mg/ml) and stored in liquid N_2. Their purity was confirmed by gas chromatographic analysis. As a precaution, a sample of the fatty acid solution was washed free of lipid peroxidation products by vortex mixing in 2 volumes of a 3 : 1 (v/v) mixture of methanol and water [11]. The heptane phase was collected, dried in a stream of N_2 gas and the fatty acid promptly redissolved in 0.12% NaOH at a concentration of 2.22 μmol/ml. This solution was administered to the suspension culture by infusion at a constant rate of 0.2 μmol/h. The syringe infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.) and syringes were maintained between 12 and 15°C throughout the 48 h period.

Membrane isolation

Cells at a density of $1 \cdot 10^6$ cells per ml were harvested from 50 ml of culture

medium and washed twice in 0.85% saline. The cell pellet was suspended in 5 ml of $1 \cdot 10^{-3}$ M zinc chloride [12], incubated for 5 min at room temperature and then reincubated an additional 30 min at 2—5°C. After incubation the cells were homogenized in a 40 ml tight fitting Dounce homogenizer (Type B, Kontes, Vineland, N.J.), and plasma membranes were isolated using the two phase dextran, polyethylene glycol polymer system of Brunette and Till [13]. To reduce nuclear contamination, the concentration of zinc chloride was decreased from $1 \cdot 10^{-2}$ M to $1 \cdot 10^{-3}$ M. Polymer phases were stored at —20°C in order to avoid irregularities in phase separation encountered with storage at higher temperatures [14]. They were thawed just prior to use and maintained at 2—5°C throughout the isolation procedure. Following homogenization, the cell suspension was centrifuged at 300 × *g* for 15 min in a refrigerated centrifuge (International Equipment Co., Boston, Mass.). The pellet was suspended by vortex mixing with 1.25 ml each of upper and lower phase solutions. This suspension was centrifuged at 8600 × *g* for 10 min in an SW-39 head of an ultracentrifuge (Model L, Beckman Instruments, Wakefield, Mass.). Material at the interface between the phases was diluted 10-fold with ice cold distilled water and centrifuged at 300 × *g* for 15 min. The resulting pellet was suspended in a volume (1 ml to 5 ml) of ice cold water convenient for subsequent analysis.

Preparation of membranes for electron microscopy

Pellets of cell membranes were fixed and processed as previously described [15]. Thin sections were cut, lightly stained with lead citrate and examined in a Philips EM 300 electron microscope.

Enzyme assay procedure

(Na^+-K^+)ATPase activity, a plasma membrane marker, was determined using the assay mixture described by Avruch and Wallach [16,17].

Cytochrome oxidase [18], a mitochondrial marker, and NADPH cytochrome *c* reductase [19], a microsomal marker, were measured as originally described.

Assay for nuclear contamination

To assess nuclear contamination of the plasma membrane fraction 5 μCi of [*Me*-^{14}C]thymidine (New England Nuclear, Boston, Mass.), specific activity 50 mCi per mmol, was administered to 50 ml of cell suspension ($5 \cdot 10^5$ cells/ml) 24 h prior to membrane isolation. Cells were harvested, rinsed 3 times in 0.85% saline and their plasma membranes isolated. Samples of whole cell homogenate and plasma membrane fractions were assayed for radioactivity in 15 ml of Aquasol (New England Nuclear) containing 1 ml of hyamine hydroxide; 0.2 ml of glacial acetic acid was added prior to counting to eliminate chemiluminescence. Samples were counted in a Beckman LS-230 liquid scintillation spectrometer. Nuclear contamination is expressed as the percent of the cell homogenate radioactivity remaining in the isolated membrane fraction.

Thin-layer chromatography

Samples of $1 \cdot 10^7$ cells or plasma membrane fractions isolated from $5 \cdot 10^7$ cells were extracted in a 2 : 1 (v/v) mixture of chloroform/methanol according to Marinetti [20] and prepared for thin-layer chromatography as

previously described [10]. The lipid was plated on 0.5 mm thick silica gel H chromatography plates impregnated with 0.11% ammonium sulfate. Each chromatogram was developed twice in the same direction using two different solvent systems [10]. Lipid fractions were visualized by spraying the chromatography plate with a 0.1% solution of 8-anilino-1-naphthalene sulfonic acid as described by Gitler [21].

Gas chromatography

Methyl esters of phosphoglyceride fatty acids were prepared as previously described [10] using the alkaline methanolysis procedure of Glass [22]. Separation of the methyl esters was achieved using a Varian Series 2440 gas chromatograph equipped with a flame ionization detector and a Hewlett Packard 3380A Integrator. The injector and detector temperatures were 220°C and the column temperature was 195°C. Fatty acids were identified by comparing their retention times with those of commercial standards (Applied Science Labs, Inc., State College, Pa.).

Protein and lipid phosphorous

Protein was measured according to the method of Lowry [23] and lipid phosphorous was determined as described by Rouser [24].

Results and Discussion

Strain L-fibroblasts supplemented with oleic or linolenic acids had markedly different phosphoglyceride fatty acid composition both from each other and from control cells (Tables I, II, III) but maintained growth rates comparable to non-supplemented cells (Table IV). The decrease in growth rate reported in a previous study was likely due to toxic effects which may result when fatty acids are administered in a single dose [8]. The present data suggest that alterations in membrane polyenoic fatty acid content over a range of from 3 to 44% do not result in changes in membrane properties which adversely affect the multiplication rate of L-fibroblasts. The maximum amount of polyenoic fatty acid which can be incorporated into strain L-fibroblast membrane phosphoglyceride without effect on viability and multiplication has yet to be determined.

There were no discernible ultrastructural changes in the plasma membrane of strain L-fibroblasts following the infusion into the culture of either oleic or linolenic acid. This is in contrast to the myelin-like figures which form after glutaraldehyde fixation in the outer mitochondrial membrane of strain L-fibroblasts supplemented with linolenic acid [15]. While some accumulation of triglyceride droplets was noted within cells of the infused culture, this was considerably less than when exogenously supplied fatty acids were administered in a single dose [25].

Electron micrographs (Fig. 1) of the plasma membrane fraction revealed material which was scroll-like and which had the typical trilaminar appearance associated with biological membranes. No nuclei or mitochondria were observed. However, there was slight contamination by endoplasmic reticulum as evidenced by the presence of small membrane vesicles studded with ribosomes (Fig. 1).

TABLE I

DISTRIBUTION OF TOTAL PHOSPHOGLYCERIDE FATTY ACIDS IN CELL HOMOGENATE AND PLASMA MEMBRANE FRACTIONS FROM NON-SUPPLEMENTED STRAIN L-FIBROBLASTS (% w/w)

The lipid from $1 \cdot 10^7$ cells or from the plasma membranes from $5 \cdot 10^7$ cells was extracted. Cell homogenate and plasma membrane phosphoglycerides were isolated, fatty acid methyl esters prepared and identified as described under Methods. An approximation of the average number of double bonds/unit mass of phosphoglyceride acyl group was calculated using the following formula: Average number of double bonds = Σ (% w/w) (no. of double bonds)/100. The data represent the average ± 1 S.D. of 3 determinations.

Fatty acid *	Cell homogenate	Plasma membrane
14 : 0	ND **	ND
16 : 0	19.8 ± 2.7	18.3 ± 2.3
16 : 1	2.2 ± 0.5	2.8 ± 0.4
18 : 0	11.4 ± 1.4	14.3 ± 0.5
18 : 1	63.9 ± 2.7	61.2 ± 3.3
18 : 2	2.6 ± 0.9	3.5 ± 1.0
18 : 3	ND	ND
Unsaturated fatty acid / Saturated fatty acid	2.2	2.07
Average no. of double bonds/fatty acid molecule	0.71	0.71

* Fatty acids are denoted by number of carbons : number of double bonds.
ND, none detected.

Enzyme markers were used to assess more quantitatively the yield of plasma membranes and contamination by other membrane elements. The specific activity of (Na^+-K^+)-dependent ATPase, a plasma membrane marker, was increased 12-fold over that of the cell homogenate. Based on the recovery of ATPase, 15% of the plasma membrane was isolated by this method. The specif-

TABLE II

DISTRIBUTION OF TOTAL PHOSPHOGLYCERIDE FATTY ACIDS IN CELL HOMOGENATE AND PLASMA MEMBRANE FRACTIONS FROM CELLS SUPPLEMENTED WITH OLEIC ACID (% w/w)

Strain L-fibroblasts grown in suspension culture containing delipidized serum were supplemented with sodium oleate [18 : 1 (*n*-9)] for 43 h at a constant rate of 0.2 μmol/h. The lipid was extracted from the same number of cells and processed as indicated in Table I. The average number of double bonds/phosphoglyceride acyl group was calculated as in Table I. The data represent the average ± 1 S.D. of 4 or 5 determinations.

Fatty acid *	Cell homogenate	Plasma membrane
14 : 0	ND	ND
16 : 0	15.0 ± 3.6	11.8 ± 2.0
16 : 1	ND	ND
18 : 0	9.1 ± 0.9	14.9 ± 0.3
18 : 1	74.0 ± 3.9	67.8 ± 2.3
18 : 2	1.8 ± 0.7	5.5 ± 0.6
18 : 3	ND	ND
Unsaturated fatty acid / Saturated fatty acid	3.1	2.7
Average no. of double bonds/fatty acid molecule	0.78	0.79

* Fatty acids are denoted by number of carbons : number of double bonds.

TABLE III

DISTRIBUTION OF PHOSPHOGLYCERIDE FATTY ACIDS IN CELL HOMOGENATE AND PLASMA MEMBRANE FRACTION FROM CELLS SUPPLEMENTED WITH LINOLENIC ACID (% w/w)

Strain L-fibroblasts grown in suspension culture supplemented with delipidized serum were supplemented with sodium linolenate [18 : 3 (n — 3)] for 48 h at a constant rate of 0.2 μmol/h. The lipid was extracted from the same number of cells and processed as indicated in Table I. The average number of double bonds per phosphoglyceride acyl group was calculated as in Table I. The data represent the average ± 1 S.D. of 4 or 5 determinations.

Fatty acid *	Cell homogenate	Plasma membrane
14 : 0	ND	ND
16 : 0	12.2 ± 0.5	17.3 ± 2.7
16 : 1	1.4 ± 0.4	1.3 ± 0.4
18 : 0	15.8 ± 0.6	16.4 ± 1.7
18 : 1	16.3 ± 1.2	16.5 ± 0.9
18 : 2	2.2 ± 0.3	5.1 ± 2.8
18 : 3	30.0 ± 1.0	21.2 ± 1.8
20 : 3	11.6 ± 0.6	15.0 ± 3.0
20 : 4	10.5 ± 0.1	7.2 ± 1.5
Unsaturated fatty acid / Saturated fatty acid	2.6	2.0
Average no. of double bonds/fatty acid molecule	1.89	1.65

* Fatty acids are denoted by number of carbons : number of double bonds.

ic activity of the mitochondrial marker, cytochrome oxidase and the microsomal marker, NADPH cytochrome c reductase, decreased by a factor of 10 or remained essentially unchanged. In addition, only 0.5% of the total radioactive thymidine initially present in the cell homogenate was recovered in the plasma membrane fraction. Based on the recovery of marker enzyme, less than 1% of the mitochondria and 4% of the endoplasmic reticulum were present as contaminants of the plasma membrane fraction.

The ratio of phospholipid to protein in the cell homogenate fractions was similar whether the cells were grown with delipidized serum or were infused with either oleic or linolenic acid and agree with previously published values for the strain L-fibroblast [26]. This value was higher in the plasma membrane fractions but, as in the cell homogenate, there was no significant difference between the cells grown under the three different conditions.

TABLE IV

GENERATION TIME OF NON-SUPPLEMENTED STRAIN L-FIBROBLASTS AND CELLS SUPPLEMENTED WITH OLEIC ACID (18 : 1) OR LINOLENIC ACID (18 : 3)

Strain L-fibroblasts were grown under the conditions described in Table II. Cell counts were obtained from triplicate flasks at 0 and 48 h, and the generation time ±1 S.D. estimated.

Fatty acid supplement *	Generation time (h)
—	22.0 ± 2.3
18 : 1	23.5 ± 0.7
18 : 3	23.8 ± 1.4

* Fatty acids are denoted by number of carbons : number of double bonds.

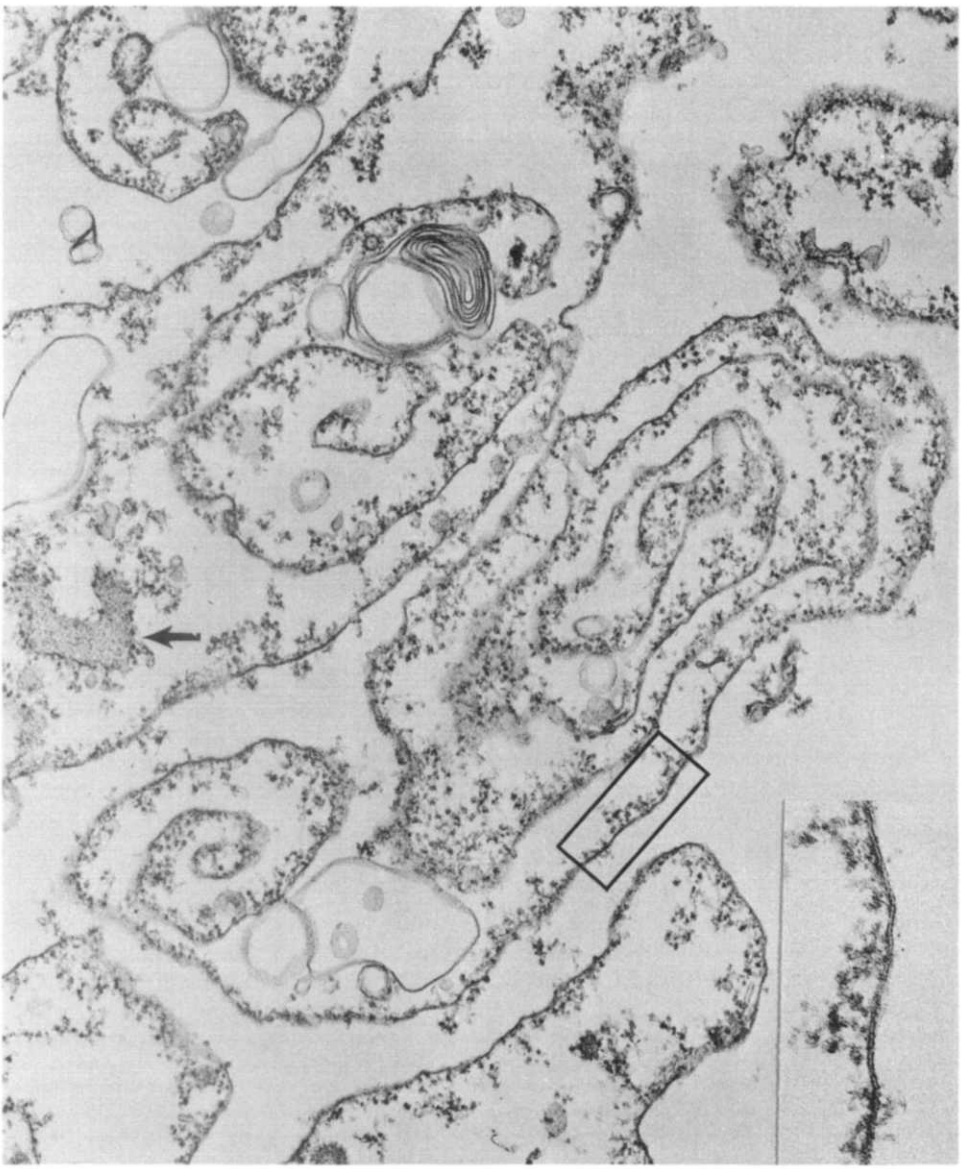

Fig. 1. Plasma membranes isolated from non-infused L-fibroblasts. These membranes have a typical bilaminar appearance (inset), and form scroll-like structures which have fibrillar material attached to one surface. Rarely a small fragment of rough endoplasmic reticulum was observed (arrow). Magnification × 36 000. Inset × 90 000.

Oleic acid is the principal phosphoglyceride fatty acid of L-fibroblasts cultured in the presence of delipidized serum (Table I). The relatively small quantity of material tentatively identified as linoleic acid (18 : 2) is likely to be derived from residual lipid (5—10%) present in the delipidized serum and not from endogenous substrates. That the latter are not utilized to a significant extent for synthesis of polyenoic fatty acids is shown by experiments in which cells grown in the complete absence of exogenous lipids, i.e. in a chemically

defined, serum-free medium, contain no fatty acid more unsaturated than oleic acid [27] and therefore lack the ability to produce quantitatively significant amounts of 18 : 2 (*n*-9). The lack of a Δ^6 desaturase in these and other transformed cell lines has been described [28].

Despite its scarcity in the medium, a greater percentage of linoleic acid was present in the phosphoglyceride of the cell membrane than in the cell homogenate of non-supplemented control cells. These results are in agreement with those from a study [26] which showed that the phospholipid of cell membrane from L-fibroblasts grown in 10% fetal calf serum (a source of exogenous lipid) contained greater amounts of polyenoic fatty acid than did the whole cell. In the presence of moderate and low levels of exogenous polyenoic fatty acid, therefore, there may be mechanisms which cause the preferential utilization of the more unsaturated phosphoglycerides for plasma membrane synthesis.

The oleic acid content of homogenate and plasma membrane fractions of cells after infusion with this fatty acid increased by 16% and 11%, respectively (Table II). This increase occurred primarily at the expense of palmitic and palmitoleic acids. The amount of exogenous oleic acid incorporated into the strain L-fibroblast phosphoglycerides is greater than it appears, however, because the exogenously supplied oleic acid suppresses the biosynthesis of fatty acids [29] including oleic acid. As a result, the exogenous oleic acid itself is substituted for endogenous oleic acid during synthesis of membrane phosphoglycerides.

The increase by 40 and 33%, respectively, in the ratio of unsaturated : saturated phosphoglyceride fatty acids in cell homogenate and plasma membrane fractions of cells supplemented with oleic acid is due both to an increase in the ratio of exogenous : endogenous fatty acids available for PL synthesis and to a decrease in the biosynthesis of the latter induced by the added oleic acid. Despite the increase above control values (Table I) the ratio of unsaturated : saturated phosphoglyceride fatty acid the average number of double bonds per acyl group was changed only slightly in both fractions after the culture was infused with oleic acid.

The most significant modification in the phosphoglyceride fatty acid composition of cell homogenate and plasma membrane fractions occurred when cells were supplemented with linolenic acid (Table III). Linolenic acid replaced oleic acid as the predominant phosphoglyceride fatty acid in both the cell homogenate and plasma membrane fraction. In addition, two other fatty acids were present in the phosphoglycerides of cells supplemented with linolenic acid. Neither of these was found in the lipids of cells from non-supplemented cultures or cultures supplemented with oleic acid, and they probably represent eicosatrienoic (20 : 3 (*n*-3)) and eicosatetraenoic (20 : 4 (*n*-3)) acids derived from the exogenous linolenic acid [8].

Although the existence of a Δ^8 desaturase in mammalian liver [30] and brain [31] has been questioned, this enzyme may be of greater significance in the lipid metabolism of L-fibroblast and other transformed cell lines in which no Δ^6 desaturase is present [28]. Additional work will be necessary to fully characterize the two fatty acids derived from linolenic acid in the present study. The total polyenoic fatty acid content of these cells was increased in the cell homogenate and plasma membrane fractions to 50 and 40%, respectively,

of the total fatty acid contents. The ratios of unsaturated : saturated fatty acids in phosphoglycerides of cell homogenates and plasma membrane fractions of cells infused with linolenic acid more nearly resembled those of control cells than those of cultures infused with oleic acid (Tables I, II and III). This resulted from the substitution of linolenic acid, and its derivatives, for oleic acid instead of for saturated fatty acids. The lower polyenoic fatty acid content of the plasma membrane was due to a decrease in the linolenic acid content of that fraction to 66% of that in the homogenate. By contrast the two fatty acids derived from linolenic acid were incorporated to the same extent into both the cell homogenate and plasma membrane phosphoglyceride fractions (Table III). As a consequence the average number of double bonds per acyl group in the plasma membrane was only 87% of that in the cell homogenate.

The distribution of fatty acids in the two principal phospholipid classes in the cell homogenate and plasma membrane fractions under the three sets of conditions employed is summarized in Tables V and VI. Palmitic acid was preferentially incorporated into the phosphatidylcholine fraction, while stearic acid was preferentially utilized for phosphatidylethanolamine biosynthesis. The average increase in monoenoic acids in the phosphatidylcholine and phos-

TABLE V

DISTRIBUTION OF STRAIN L-FIBROBLAST PHOSPHOGLYCERIDE FATTY ACIDS IN CELL HOMOGENATE PHOSPHATIDYLCHOLINE (PC) AND PHOSPHATIDYLETHANOLAMINE (PE) OF NON-SUPPLEMENTED CONTROL CELLS AND CELLS SUPPLEMENTED WITH OLEIC OR LINOLENIC ACIDS (% w/w)

Strain L-fibroblasts in suspension were grown and supplemented as indicated in Table II. Phosphatidylcholine and phosphatidylethanolamine were isolated from the lipid extract from $1 \cdot 10^7$ cells. Fatty acid methyl esters of phosphatidylcholine and phosphatidylethanolamine acyl groups were prepared and identified as described under Methods. The average number of double bonds per phosphoglyceride fatty acid molecule was calculated as in Table III. The data represent the average ±1 S.D. of three determinations.

Fatty acid *	Control non-supplemented		Oleic acid supplemented		Linolenic acid supplemented	
	PC	PE	PC	PE	PC	PE
14 : 0	ND	ND	ND	ND	ND	ND
16 : 0	21.5 ± 0.6	8.7 ± 0.4	13.2 ± 1.2	6.1 ± 1.4	17.3 ± 1.4	5.2 ± 0.5
16 : 1	4.7 ± 0.2	3.3 ± 0.2	1.4 ± 0.9	1.1 ± 0.2	1.8 ± 0.1	1.1 ± 0.3
18 : 0	4.6 ± 0.2	18.9 ± 1.6	3.1 ± 0.2	16.4 ± 1.3	7.6 ± 0.4	25.6 ± 0.6
18 : 1	67.2 ± 1.0	64.9 ± 0.7	80.1 ± 2.4	72.0 ± 1.3	16.5 ± 0.8	20.5 ± 1.5
18 : 2	2.0 ± 0.1	3.6 ± 0.5	2.1 ± 0.3	4.3 ± 1.4	2.0 ± 0.3	2.4 ± 0.4
18 : 3	ND	0.5 ± 0.1	ND	ND	34.8 ± 1.4	26.7 ± 0.2
20 : 3	ND	ND	ND	ND	10.4 ± 0.1	7.6 ± 0.4
20 : 4	ND	ND	ND	ND	9.6 ± 1.1	10.8 ± 0.6
Unsaturated fatty acid / Saturated fatty acid	2.8	2.6	5.1	3.4	3.0	2.2
Average no. of double bonds/fatty acid molecule	0.76	0.78	0.84	0.82	1.96	1.72
20 : 3 + 20 : 4/18 : 3	—	—	—	—	0.57	0.69

* Fatty acids are denoted by number of carbons : number of double bonds.

TABLE VI

DISTRIBUTION OF STRAIN L-FIBROBLAST PHOSPHOGLYCERIDE FATTY ACIDS IN PLASMA MEMBRANE PHOSPHATIDYLCHOLINE (PC) AND PHOSPHATIDYLETHANOLAMINE (PE) OF CONTROL, OLEIC AND LINOLENIC ACID SUPPLEMENTED CELLS (% w/w)

Plasma membranes were isolated from $5 \cdot 10^7$ cells grown and supplemented as indicated in Table II. Phosphatidylcholine and phosphatidylethanolamine were isolated from the plasma membrane, and their fatty acid methyl esters were prepared and identified as described under Methods. The average number of double bonds/phosphoglyceride fatty acid molecule was calculated as in Table III. The data represent the average ±1 S.D. of three determinations.

Fatty acid *	Control non-supplemented		Oleic acid supplemented		Linolenic acid supplemented	
	PC	PE	PC	PE	PC	PE
14 : 0	ND	ND	ND	ND	ND	ND
16 : 0	26.0 ± 2.0	10.8 ± 1.8	12.7 ± 0.4	8.0 ± 1.3	23.2 ± 3.1	16.8 ± 2.9
16 : 1	5.0 ± 0.4	4.4 ± 0.7	2.9 ± 0.5	6.2 ± 2.6	2.2 ± 0.3	4.0 ± 1.1
18 : 0	7.6 ± 0.3	19.2 ± 1.2	3.9 ± 0.8	13.3 ± 3.1	9.0 ± 1.4	19.3 ± 2.0
18 : 1	58.0 ± 1.2	59.4 ± 3.1	75.7 ± 1.8	70.2 ± 0.8	20.7 ± 3.7	31.1 ± 2.8
18 : 2	2.6 ± 0.8	5.4 ± 0.6	4.4 ± 1.1	2.2 ± 0.2	3.5 ± 1.8	5.4 ± 1.0
18 : 3	0.4 ± 0.3	0.8 ± 0.2	0.3 ± 0.2	ND	23.6 ± 3.1	12.2 ± 2.5
20 : 3	ND	ND	ND	ND	11.3 ± 3.2	6.3 ± 1.1
20 : 4	ND	ND	ND	ND	6.4 ± 1.3	4.7 ± 1.2
Unsaturated fatty acid / Saturated fatty acid	1.94	2.3	5.0	3.7	2.1	1.8
Average no. of double bonds/fatty acid molecule	0.67	0.77	0.88	0.81	1.60	1.20
20 : 3 + 20 : 4/18 : 3	—	—	—	—	0.75	0.90

* Fatty acids are denoted by number of carbons : number of double bonds.

phatidylethanolamine of plasma membrane of cells infused with oleic acid was slightly more than that in the cell homogenate. In both phosphoglyceride fractions a decrease in the saturated fatty acids accompanied the increase in oleic acid content.

Infusion of linolenic acid into the culture resulted in a marked increase in the polyenoic fatty acid content in the phosphatidylcholine and phosphatidylethanolamine fraction of the plasma membrane and homogenate (Tables V and VI). In both phospholipid classes the increase in linolenic acid was less in the plasma membrane than in the cell homogenate. The increase in the average number of double bonds per phosphatidylcholine acyl group was similar in homogenate and plasma membrane fractions (2.40-fold and 2.58-fold, respectively). In contrast, the average number of double bonds/acyl group in the phosphatidylethanolamine fraction of the plasma membrane was reduced when compared to the whole cell (1.58-fold vs. 2.20-fold). This resulted from a decrease in linolenic acid and an increase in oleic acid content of plasma membrane phosphatidylethanolamine when compared to cell homogenate phosphatidylethanolamine.

The differences in phosphoglyceride fatty acid composition which exist between the cell homogenate and plasma membrane fractions, following the infusion of exogenous fatty acid into the culture, suggest that certain phosphoglyceride species may be selected for or excluded from the plasma mem-

brane on the basis of their unsaturation. This non-random distribution of phosphoglycerides might arise by selection during the synthesis of new surface membrane during the infusion and/or after infusion by the rapid exchange of intact phosphoglyceride molecules, or their component fatty acids, between surface and intracellular membranes. Such a mechanism might be one means by which a cell could regulate the physical and biochemical properties of its plasma membrane when exposed to large amounts of polyenoic fatty acid. The increase in the saturated fatty acid content of phosphoglycerides of cells infused with linolenic acid might be of significance in modulating membrane fluidity [5]. However, it is also possible that it is a consequence of the passive accumulation of endogenous saturated fatty acids, which, in the absence of exogenous unsaturated fatty acids would normally be desaturated to form oleic acid.

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