review

Membrane lipid composition and cellular function

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Abstract Membrane fatty acid composition, phospholipid composition, and cholesterol content can be modified in many different kinds of intact mammalian cells. The modifications are extensive enough to alter membrane fluidity and affect a number of cellular functions, including carrier-mediated transport, the properties of certain membrane-bound enzymes, binding to the insulin and opiate receptors, phagocytosis, endocytosis, depolarization-dependent exocytosis, immunologic and chemotherapeutic cytotoxicity, prostaglandin production, and cell growth. The effects of lipid modification on cellular function are very complex. They often vary from one type of cell to another, and they do not exert a uniform effect on all processes in a single cell line. Therefore, it is not yet possible to make any generalizations or to predict how a given system will respond to a particular type of lipid modification. Many of the functional responses probably are caused directly by the membrane lipid structural changes, which affect either bulk lipid fluidity or specific lipid domains. The conformation or quaternary structures of certain transporters, receptors, and enzymes probably are sensitive to changes in the structure of their lipid microenvironment, leading to changes in activity. Prostaglandin production is modulated by the availability of substrate fatty acids stored in the membrane phospholipids, but the underlying chemical mechanism still involves a change in membrane lipid structure. While this is the most likely mechanism, the possibility that the membrane lipid compositional change is an independent event that occurs concurrently but is not causally related to the functional perturbations also must be considered. —**Spector, A. A., and M. A. Yorek.** Membrane lipid composition and cellular function. *J. Lipid Res.* 1985. **26:** 1015–1035.

Supplementary key words fatty acid • phospholipids • cholesterol • membrane fluidity • membrane transport • enzymes • receptors • prostaglandins • cell culture

I. BACKGROUND

Mammalian cell membranes consist of a lipid bilayer composed primarily of phospholipids and cholesterol. Proteins that have important cellular functions, such as receptors, transporters, and enzymes are embedded in the lipid bilayer. The dynamic state of the lipids in the bilayer was described in 1972 by Singer and Nicolson (1) as one of the fundamental tenents of the fluid mosaic model of membrane structure. A basic question raised by this formulation was whether interactions with lipids contained in the bilayer can modulate the activity of membrane proteins.

At the time that the fluid mosaic model was formulated, evidence was becoming available that membrane lipids can influence the function of certain membrane proteins. For example, it already was known that when human erythrocyte membranes were exposed to phospholipase A_2, which hydrolyzes fatty acyl groups from the *sn*-2 position of glycerophospholipids, glucose transport was reduced by 75% (2). In addition the Na^+ + K^+-ATPase activity of the erythrocyte membrane was decreased. Incubation with phospholipases C or D, which remove the phospholipid head groups located on the surface of the membrane lipid bilayer, had much less effect on glucose transport and Na^+ + K^+-ATPase activity. While such studies established that the structure of the lipid bilayer can influence the activity of a membrane protein, they provided no information as to whether such effects actually occurred in an intact cell. From the standpoint of cellular physiology and metabolic regulation, the critical questions were whether changes in membrane lipid composition actually can occur in a living cell and if so, whether the changes are of sufficient magnitude to influence membrane function. An extensive amount of information regarding these issues has been obtained during the last decade, much of it from studies with mammalian cells in culture.

The purpose of this review is to briefly describe the types of changes in membrane lipid composition that can be produced under biological conditions and their functional consequences, with emphasis on data obtained from cell culture studies. A comprehensive review dealing with lipid effects on membrane function was prepared recently by Stubbs and Smith (3). Additional reviews are available on related topics, including the effect of lipids on membrane bound enzymes (4), membrane asymmetry (5, 6), the dynamics of membrane structure (7) and lipid metabolism in cultured cells (8).

Abbreviations: ESR, electron spin resonance; S, membrane order parameter; τ_0, probe motion parameter; 12-NS, 12-nitroxide stearic acid; 5-NS, 5-nitroxide stearic acid; DPH, 1,6-diphenyl-1,3,5-hexatriene; $\bar{\eta}$, membrane viscosity; PGE_1, prostaglandin E_1; PGI_2, prostaglandin I_2; TXA_2, thromboxane A_2.

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A. Cultured cells as a model system

A number of early observations indicated that it might be possible to produce membrane lipid modifications in cultured cells. Mammalian cells were found to readily take up lipids contained in the culture medium, with much of the fatty acid and cholesterol taken up being utilized for membrane synthesis (8). When the culture medium contains an adequate supply of lipids, fatty acid and cholesterol synthesis are suppressed, and most of the cellular lipids are derived from the material that is taken up from the medium. These findings suggested that it might be possible to influence the lipid composition of cells by controlling the type of lipids added to the culture medium. As an extension of this work, it was found that certain cells can be propagated in a serum-free medium. There was a concern initially that such cultures might stop growing when either linoleic acid (18:2)[1] or related essential polyunsaturated fatty acids became depleted. However, this proved not to be the case (9). Certain cells grown under these conditions do not have measurable amounts of polyunsaturated fatty acids in membrane phospholipids; instead, they contain large quantities of two monounsaturates that can be produced biosynthetically, oleic (18:1) and palmitoleic (16:1) acids (10). By contrast, when these cells are grown in the presence of serum they normally contain 20–30% polyunsaturated fatty acids (8). Considering these two cases as extremes, it became clear that variants of a cell line having widely differing membrane fatty acid compositions could be produced simply by altering the lipid content and composition of the culture medium.

II. METHODS FOR MODIFYING LIPID COMPOSITION

Most membrane lipid modifications in cultured cells are produced by supplementing the medium with specific lipids, usually bound to albumin or serum proteins. It also is possible to modify the phospholipid composition and cholesterol content of cells by incubation with different types of liposomes.

A. Fatty acid and serum supplements

The first studies with lipid supplements were done with mouse LM cells, a variant of the L fibroblast that can grow in a serum-free medium. By adding *dl*-desthiobiotin to inhibit fatty acid synthesis together with various fatty acid esters of Tween, large differences in the fatty acid composition of the LM cell phospholipids were produced (11). Using this approach, the percentage of saturated fatty acids in the cell phospholipids could be varied from 28 to 85% (12). In other experiments the linoleic acid content was raised from an undetectable amount to 37% of the phospholipid fatty acyl groups by supplementing a serum-free medium with linoleic acid bound to albumin (13). Phospholipid fatty acyl modifications also were produced in 3T3 cells by growing them in a culture medium containing lipid-depleted serum, supplemented with specific fatty acids (14).

Although useful in certain cases, these methods are limited in that many diploid cells cannot be grown in adequate amounts in either a serum-free medium or a medium that contains lipid-extracted serum. However, subsequent studies with human skin fibroblasts indicated that it is not essential to remove the serum lipids from the culture medium. Extensive modifications in the fibroblast fatty acyl composition were produced by adding specific fatty acids to the usual growth medium containing 10% fetal bovine serum. The cultures continued to grow normally under these conditions, and large quantities of lipid-modified fibroblasts could be produced (15). In addition it was observed that fibroblasts can be modified even after the cultures became confluent (15). Supplementation of the medium with specific fatty acids is currently the most widely used method for producing membrane lipid modifications in cultured cells.

Fairly extensive phospholipid fatty acyl modifications also can be produced in certain cells by replacing fetal bovine serum with horse serum in medium (16). These differences apparently result from the fact that horse serum contains 65% more polyunsaturated fatty acid than fetal bovine serum (16).

B. Phospholipids

Cultured cells can take up large quantities of intact phospholipids from unilamellar vesicles without affecting cell viability (17). About 20–30% of the uptake remains associated with the plasma membrane. At 37°C, the predominant process that occurs is fusion of the vesicle with the cell membrane (18). If the incubation is carried out at 2°C, however, phospholipid uptake occurs through an exchange between the outer leaflet of the vesicle and the cell membrane (19). Under these conditions the phospholipids that enter the plasma membrane are associated initially with the outer leaflet of the lipid bilayer. Phospholipids that have been incorporated into cell membranes by incubation with liposomes include phosphatidylethanolamine (20) and a variety of phosphatidylcholines including egg yolk, dioleoyl, dipalmitoyl and dimyristoyl (21). Liposomes also can be employed to insert fluorescent analogues of phosphatidylcholine, phosphatidylethanolamine, and phosphatidic acid into the plasma membrane of intact cells (22–24). These compounds contain the fluorescent N-4-nitrobenzo-2-oxa-1,3-diazole aminocaproyl group in

[1] Fatty acids are abbreviated as number of carbon atoms:number of double bonds. Thus, 18:2 contains 18 carbon atoms and two double bonds.

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the *sn*-2 position. They are presented to cells as components of small, unilamellar dioleoyl phosphatidylcholine vesicles. Based upon the distribution of these analogues as visualized by fluorescence microscopy, it appears that phospholipids taken up from liposomes are incorporated into the nuclear membrane, mitochondria, and Golgi apparatus if the cultures are maintained at 37°C.

Liposomes have not been used extensively to produce membrane lipid modifications. A potential advantage of this method is that changes can be produced in a single class of phospholipids, whereas all of the glycerophospholipid classes are modified to some extent when either fatty acid or serum supplements are used. Another advantage of using liposomes is that the fatty acyl modifications can be confined initially to either the *sn*-1 and *sn*-2 positions of the glycerophospholipids; this cannot be precisely controlled with fatty acid or serum supplements.

Extensive modifications also can be produced in the composition of the cellular phospholipids by altering the availability of the compounds that are used to form the polar head group. Most of this work has been done with suspension cultures of LM cells (13, 20). The approach is to replace choline in the growth medium with various analogues, including ethanolamine, N-methyl ethanolamine, N,N-dimethyl ethanolamine, 2-amino-2-methyl-1-propanol, 2-amino-1-butanol, or 3-aminopropanol. A considerably altered phospholipid composition occurs in all subcellular membrane fractions within 3 days (20). The phosphatidylcholine content, which normally comprises 48% of the phospholipids, can be reduced to as low as 12%, and the phosphatidylethanolamine content reduced from 28% to 13%. These polar head groups are replaced by choline and ethanolamine analogues, the maximum amounts being 49% dimethyl-, 37% monomethyl-, and 34% aminobutanol glycerophospholipid. Little change occurs in the phosphatidylinositol, phosphatidylserine, or cardiolipin content, and the ratio of neutral to acidic phospholipids remains fairly constant under these conditions (20). Increases in sphingomyelin and lysophosphatidylcholine occur, but these phospholipids are present in only relatively small amounts. These changes are accompanied by a 30% decrease in the sterol to phospholipid molar ratio of the plasma membrane. Therefore, any functional changes that occur cannot be attributed with certainty only to the change in phospholipid head group composition.

C. Sphingolipids

Sphingomyelin can be taken up by cultured human skin fibroblasts (25). A fluorescent ceramide analogue is taken up by Chinese hamster lung fibroblasts, converted into sphingomyelin or glucocerebroside and incorporated into membranes (26). Gangliosides added to the medium are incorporated into the plasma membrane of developing neurons (27), 3T3 cells (28), and neuroblastoma cells (29). Increasing amounts of the ganglioside are taken up as the extracellular concentration is raised. These approaches can be employed to examine the effects of sphingolipid content and composition on cellular and membrane function.

D. Cholesterol

By incubating erythrocytes with phospholipid vesicles containing a large amount of cholesterol, it is possible to obtain membranes having a cholesterol to phospholipid molar ratio as high as 2.7 (30). Normally, the molar ratio of cholesterol to phospholipid in the erythrocyte membrane is about 0.8–0.9. Through similar approaches, enrichment of the plasma membrane with cholesterol has been obtained in platelets (30), lymphocytes (31), and ascites lymphoma cells (32). Conversely, by incubating erythrocytes with cholesterol-free phosphatidylcholine vesicles, cholesterol can be removed from the membrane and the molar ratio of cholesterol to phospholipid can be reduced to as low as 0.4 (33, 34). Likewise, cholesterol efflux from L cells occurs when they are incubated with albumin-phospholipid complexes (35). The maximum efflux of cholesterol takes place when a saturated phosphatidylcholine containing 18-carbon atom fatty acyl chains is utilized. Cholesterol also can be removed from human skin fibroblasts by incubation with heptane-extracted (cholesterol-depleted) high density lipoproteins (36).

In LM cells, cholesterol depletion is accompanied by an increase in the 18:1 to palmitic acid (16:0) ratio in membrane phospholipids (37). Therefore, more than one variable may be affected when the membrane cholesterol content is modified in an intact, nucleated cell. Such ancillary effects should be considered before attributing any functional change specifically to an increase or decrease in membrane cholesterol content.

The converse of this effect does not occur; changes in fatty acid composition are not accompanied by appreciable changes in the membrane cholesterol content. This was demonstrated first in Ehrlich ascites cells, where a wide range of fatty acid replacements did not produce any consistent change in the plasma membrane cholesterol content (38). There also was no change in the membrane cholesterol content when the fatty acid composition of human skin fibroblasts was modified extensively (15). These findings suggest that there is not always a linkage between membrane fatty acid composition and cholesterol content.

E. Dietary lipid and ascites tumor cells

Ascites tumor cell lines, which grow as suspensions in the peritoneal cavity of rodents, are advantageous because they can be grown inexpensively in very large amounts. Enough material can be obtained routinely to make mem-

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brane preparations equal in amount to those ordinarily prepared from rat liver. The membrane fatty acid composition of the ascites cells can be modified by feeding the animals diets that are enriched with either saturated or polyunsaturated fat. Extensive plasma membrane fatty acid modifications have been produced in two ascites cell lines using this approach, the Ehrlich ascites cell (39) and the L1210 leukemic lymphoblast (40).

III. FATTY ACID MODIFICATIONS

The fatty acyl composition of many different types of cultured cells has been modified (11-15, 36, 41-59) as listed in **Table 1**. In Ehrlich ascites cells and human skin fibroblasts, these modifications are not accompanied by any changes in the phospholipid head group composition or membrane cholesterol to phospholipid molar ratio (15, 38). This suggests that the mechanism is fatty acyl substitution. However, phospholipid and cholesterol measurements have not been made in many of the modified systems or for all of the different fatty acyl enrichments that have been produced. Therefore, it cannot be stated with certainty that in every case the only membrane perturbations are the phospholipid fatty acyl substitutions.

A. Phospholipids

A representative sampling of the types of modifications in phospholipid fatty acyl composition that can be produced either by adding nontoxic amounts of free fatty acid to culture media containing 10% fetal bovine serum or by changing the type of serum is shown in **Table 2**. There is considerable variation in the extent to which different fatty acids can be increased. This is demonstrated by the data for the bovine pulmonary artery endothelial cells supplemented with four different fatty acids. Supplementation with palmitic acid increased the phospholipid 16:0 content and total saturated fatty acid content by only a small amount. With oleic acid, however, the 18:1 content of the endothelial phospholipids increased by 60%. To compensate for this increase, there was a large decrease in the saturated fatty acid content of the phospholipids.

A very large increase in the 18:2 content of the phospholipids occurred when the endothelial cultures were supplemented with linoleic acid. This was accompanied by a 60% decrease in the 18:1 content, together with a small increase in saturated fatty acids. The enrichment with 18:2 did not produce any increase in the 20:4 content of the endothelial phospholipids, the latter actually decreased by 30%. Instead, there was an accumulation of eicosadienoic acid (20:2), the elongation product of 18:2. The failure of the endothelium to accumulate 20:4 under these conditions is not due to any intrinsic inability of the cells to store an increased amount of this fatty acid. When the endothelial cultures are supplemented directly with arachidonic acid, the 20:4 content of the phospholipids doubles. In this case a large increase in docosatetraenoic acid (22:4) also occurs, but there is no increase in docosa-

TABLE 1. Fatty acid modifications in cultured cells

Cells		Fatty Acid Enrichment[a]	References
Type	Line		
Adipocyte	3T3-L1	18:1, 18:2, 20:4	41
Ascites tumor	Ehrlich	14:0, 15:0, 18:1, 18:2, 18:3, 12-Me-14:0[b], 18:2*t*,*t*[c]	36
Endothelium	Human umbilical vein	18:1, 18:2	42
	Bovine pulmonary artery	18:0, 18:2, 20:3, 20:4	43
Fibroblast	IMR-90 lung	18:1, 18:2	15
	Human skin	16:0, 18:1, 18:2, 18:3, 20:4	15
	L	18:1, 18:2	44
	LM	15:0, 16:0, 17:0, 19:0, 20:0, 21:0, 18:1, 18:2, 18:3, 20:4	11-13, 45
	3T3	16:0, 17:0, 18:0, 18:1, 18:2	14, 46
Hematopoietic	Friend erythroleukemia	18:1, 18:2	47
	Human platelets	18:2, 20:4	48
	Splenic lymphocytes	14:0, 16:0, 17:0, 18:0, 18:1, 18:2	49
Kidney	MDCK tubular epithelium	18:1, 18:2, 20:4	50
Liver	Hepatoma 7777	18:1, 18:2	51
Macrophage	Peritoneal	19:0, 18:1	52
Neural	Mouse fetal brain	18:2, 18:3, 20:4, 22:6	53
	Neuroblastoma M1	18:2, 18:3, 20:4, 22:6	54
	PC-12 pheochromocytoma	16:0, 18:0, 18:1, 18:2, 20:4	55
	Y79 retinoblastoma	16:0, 18:1, 18:2, 18:3, 20:4, 22:6	56-58
Ovary	CHO	16:0, 18:0, 18:1, 18:2	59

[a] Supplemental fatty acid added to the growth medium.
[b] 12-Methyl myristic acid.
[c] Linolelaidic acid.

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TABLE 2. Effects of lipid supplements on phospholipid fatty acid composition of cultured cells

Cells	Lipid Supplement	Phospholipid Fatty Acid Composition[a]										
		16:0	16:1	18:0	18:1	18:2	18:3	20:2	20:4	20:5	22:4	22:6
		%										
Endothelial[b]	None[c]	20.2	3.2	21.3	27.5	2.6	ND[d]	0.3	8.9	ND	1.7	3.7
	Palmitic acid[e]	24.8	4.3	22.0	25.8	2.9	ND	0.9	6.3	ND	0.8	2.6
	Oleic acid[e]	14.0	3.7	13.8	43.6	2.2	ND	0.1	7.7	ND	1.7	2.4
	Linoleic acid[e]	17.6	2.2	16.3	18.0	19.5	ND	5.0	6.1	ND	1.5	2.7
	Arachidonic acid[e]	20.3	2.6	16.6	17.1	2.0	ND	ND	17.5	ND	13.0	2.6
3T3	None[c]	18.4	6.1	15.3	31.4	2.3	ND	ND	3.7	ND	1.0	1.2
	Linoleic acid[e]	21.0	4.9	17.2	18.6	5.0	ND	ND	14.6	ND	3.5	1.5
Y79 retinoblastoma	None[c]	23.9	9.8	14.2	20.2	1.5	ND	ND	7.9	0.4	ND	4.3
	Linolenic acid[f]	22.8	7.9	15.2	16.6	1.6	2.8	ND	9.6	2.9	ND	6.3
MDCK	Fetal bovine serum	13.8	4.1	16.3	45.1	2.0	0.2	ND	4.2	ND	ND	1.0
	Horse serum[g]	14.1	1.8	19.5	26.9	16.2	0.8	ND	9.0	ND	ND	0.1

[a]The values do not add up to 100% because some fatty acids were not identified and minor components are not listed.
[b]Bovine pulmonary artery endothelial cells.
[c]Medium contained 10% fetal bovine serum.
[d]Not detected.
[e]One hundred μM fatty acid added to 10% fetal bovine serum.
[f]Thirty μM fatty acid added to 10% fetal bovine serum.
[g]Ten percent horse serum in place of fetal bovine serum.

pentaenoic acid (22:5). Like endothelial cells, human skin fibroblasts do not become enriched in 20:4 when they accumulate 18:2; the 20:4 content of the fibroblast phospholipids also decreases (15). Furthermore, human platelets (48), as well as many continuously cultured cell lines (43, 45, 47, 51), do not show any increase in 20:4 content when they become enriched in 18:2. All of these cells apparently express a very low 6-desaturase activity when they are grown in a lipid-rich medium (60, 61), and in certain cases the 18:2 and 20:2 that accumulate displace some 20:4 from the phospholipids.

As opposed to these results, supplementation of 3T3 cells with linoleic acid produces a fourfold increase in the 20:4 content of the phospholipids. Some increase in 22:4 also occurs. Other cells that become enriched with 20:4 when they are supplemented with linoleic acid include the human Y79 retinoblastoma (57), 3T3-L1 (41), and C1300 neuroblastoma (54). All of these cells retain 6- and 5-desaturase activity under ordinary culture conditions. Furthermore, when the Y79 retinoblastoma cells are enriched with linolenic acid (18:3), the eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) contents of the phospholipids increase. This is the only continuously cultured cell line known to retain appreciable amounts of 4-desaturase activity, in addition to 6- and 5-desaturase activities.

Extensive fatty acyl modification of the cell phospholipids also can be produced by changing the type of serum present in the culture medium. Results for MDCK cells are presented in Table 2. When fetal bovine serum is replaced by horse serum, which has a high linoleic acid content (8), there is an eightfold increase in the 18:2 content of the cell phospholipids.

Although not shown in this table, it is possible to enrich cells with fatty acids that either are not found physiologically or are not present in appreciable amounts. These include odd carbon atom fatty acid (11, 38), branched chain fatty acids (38), *trans*-unsaturated fatty acids (38, 52), cyclopentenyl fatty acids (62), and fluorescent fatty acids containing conjugated double bonds (48, 63).

The extent of modification depends on the time of exposure to the supplemental fatty acid and its concentration. This is illustrated in **Fig. 1** for the Y79 retinoblastoma cells supplemented with docosahexaenoic acid. The

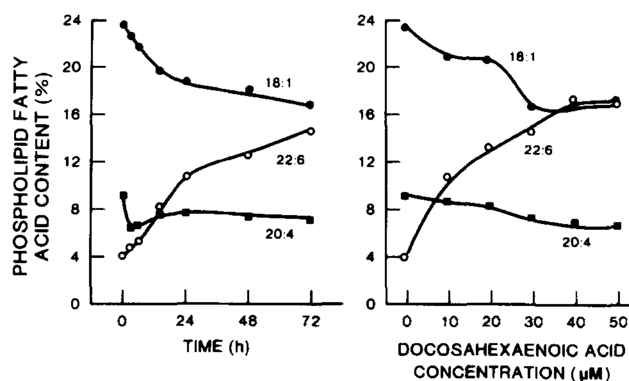

Fig. 1. Time and fatty acid concentration dependence of phospholipid fatty acyl modifications. These results were obtained with human Y79 retinoblastoma cells during log phase growth in a medium containing 10% fetal bovine serum. In the time-dependent experiment, the medium contained 30 μM supplemental docosahexaenoic acid. In the concentration-dependent experiment, the time of exposure was 72 hr.

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largest phospholipid fatty acyl compositional changes occur during the first 48 hr. In 72-hr exposures, the maximum enrichment with 22:6 is produced with a supplemental fatty acid concentration of less than 40 μM. The highest enrichments that have been produced using fatty acid supplementation of a medium containing serum is 62% 18:1 with oleic acid and 46% 18:2 with linoleic acid (51). Even in these instances, the phospholipids still contain a mixture of fatty acyl groups, not a single component as is often the case in reconstituted systems.

All of the glycerophospholipid fractions become enriched when supplemental fatty acid is added, but to different extents (42, 56, 64, 65). **Table 3** contains representative results, obtained from Y79 retinoblastoma cells supplemented with various fatty acids. With oleic acid, the ethanolamine glycerophospholipids are enriched in 18:1 to the highest level, the serine glycerophospholipids show the largest degree of enrichment, and the inositol glycerophospholipids are only slightly enriched. By contrast, the inositol phospholipids show the highest enrichment in 20:4 when supplemental arachidonic acid is present. The ethanolamine and serine phospholipids have the highest enrichments with 22:6 when the cultures are supplemented with docosahexaenoic acid.

1. Essential fatty acid deficiency. When cells are grown for long periods in a fatty acid-free medium, they lose almost all of their 18:2 and 20:4 contents (9). Yet, most cell lines do not accumulate the eicosatrienoic acid associated with essential fatty acid deficiency, 20:3 (n-9), under these conditions. When the $HSDM_1C_1$ cell line which originates from a fibrosarcoma becomes depleted of 20:4, however, 20:3 (n-9) accumulates and reaches a level of up to 6.6% of the total fatty acids (66). Phosphatidylinositol and phosphatidylcholine are the two phospholipid fractions in which 20:3 (n-9) accumulates in $HSDM_1C_1$ cells. Furthermore, 20:3 (n-9) accumulates in 3T3-L1 and 3T3 cells when they are grown in 10% fetal bovine serum. This occurs after the cultures become confluent (41). It has been observed in both the preadipocyte and differentiated forms of 3T3-L1, where 20:3 (n-9) can accumulate to levels of up to 9% of the phospholipid fatty acids. The ethanolamine and inositol glycerophospholipid fractions are the phospholipids most highly enriched in 20:3 (n-9) in 3T3-L1 cells. When growing 3T3-L1 cultures are supplemented with linoleic or arachidonic acid, the build-up of 20:3 (n-9) is prevented. Likewise, if confluent cultures already containing 20:3 (n-9) are supplemented with arachidonic acid, the 20:3 (n-9) content of the phospholipids decreases (41).

B. Neutral lipids

Cellular neutral lipids also become enriched with supplemental fatty acid. The only fraction that has been studied extensively is the triglyceride. In bovine aortic endothelial cells (67) and hepatoma 7777 cells (51), the extent of enrichment with 18:1, 18:2, and 20:4 often is greater in the triglycerides than in the phospholipids. A build-up of triglyceride mass occurs when cultures are exposed to supplemental fatty acid (15, 67, 68). Therefore, the change in fatty acyl composition is due to a net accumulation of triglyceride (67), not fatty acyl substitution in a fixed amount of lipid as occurs in the phospholipids.

C. Reversion of modified fatty acid composition

It is important to know the stability of any lipid modification in order to properly design and interpret functional studies. For example, suppose one wishes to examine the effect of fatty acid compositional changes on the induction of a particular enzyme. Two populations of cells are pre-

TABLE 3. Fatty acyl enrichment of phospholipid classes in Y79 retinoblastoma cells[a]

Phospholipid Fraction	Fatty Acyl Composition[b]					
	18:1		20:4		22:6	
	Control	Enriched with Oleic Acid	Control	Enriched with Arachidonic Acid	Control	Enriched with Docosahexaenoic Acid
				%		
Choline	25.9	39.2	6.1	15.7	2.3	17.3
Ethanolamine	31.7	49.6	13.4	27.2	5.1	30.8
Inositol	15.0	17.0	28.5	39.3	ND[c]	3.3
Serine	18.5	30.2	5.5	14.6	8.4	30.7

[a]The supplemental fatty acid concentration was 30 μM in a medium containing 10% fetal bovine serum, and the time of incubation was 72 hr.
[b]Complete fatty acid compositional results were obtained for each glycerophospholipid fraction, but for clarity only results for 18:1, 20:4 and 22:6 are listed. The control cultures were grown in a medium containing 10% fetal bovine serum but no supplemental fatty acid. In each case, the enriched cultures were supplemented with the same fatty acid for which the data are shown. For example, the data for 18:1 with enriched cultures were obtained from cells grown in a medium supplemented with oleic acid; the data for 20:4 with enriched cultures were obtained from cells supplemented with arachidonic acid, etc.
[c]Not detected.

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pared, one grown in a medium supplemented with a polyunsaturate such as linoleic acid and the other in a medium supplemented with a saturate such as palmitic acid. After the desired compositional changes are produced, both sets of cultures must be transferred to the same medium in order to be certain that any effect on enzyme induction is due to the cellular lipid modification, not to a difference in the medium to which the cultures are exposed during the induction period. Often, both cultures are transferred to a routine maintenance medium containing either 10% serum or lipid-depleted serum. If a short functional measurement is made, such as a comparison of the initial rate of amino acid uptake (69) or ionophore-stimulated prostaglandin production (46), it is unlikely that the cellular lipid modification will revert during the period of measurement. Where a longer test period is necessary, such as in the hypothetical enzyme induction study, this can become a serious problem.

Studies with Ehrlich ascites cells indicate a rapid turnover of newly incorporated phospholipid fatty acyl groups (70, 71), suggesting the possibility of rapid reversion. A substantial degree of phospholipid fatty acid turnover also occurs in L cells, especially when lipids are added to the culture medium (72–74). Furthermore, L1210 leukemia cells enriched in polyunsaturated fatty acids exhibit reductions in the phospholipid content of 18:2 from 17 to 10% and of 22:5 from 12 to 8% within 24 hr after transfer to a medium containing 20% fetal bovine serum (75). Human umbilical vein endothelial cells also exhibit reductions in the phospholipid content of 18:2 from 27 to 12% during the first 48 hr after transfer to a medium containing 20% fetal bovine serum (42).

Fig. 2 illustrates typical reversion results obtained with human skin fibroblasts (68). Following enrichment, the fibroblast phospholipids contained 25% 18:2. The cultures then were maintained for up to 7 days in one of three media. Those transferred to a medium containing 10% fetal bovine serum exhibited very little decrease in phospholipid 18:2 content during the first 24 hr. Subsequently, the 18:2 content declined to 11.5% after 7 days. Cultures transferred to a medium containing 10% lipid-depleted serum exhibited a somewhat more rapid decline in 18:2 content, including a decrease from 25 to 22% after 24 hr. The most rapid decrease occurred when the maintenance medium contained 10% fetal bovine serum supplemented with 75 μM oleic acid, in which case the phospholipid 18:2 content decreased to 16% after only 6 hr, a loss of 35% of the initial 18:2 enrichment. These data indicate that while reversion occurs, the rate depends on the type of maintenance medium to which the cultures are transferred. If this medium contains a supplemental fatty acid, reversion occurs so rapidly that long-term functional tests are precluded.

The triglyceride accumulation reverts even more rapidly after removal of the supplemental fatty acid from the culture medium. In human skin fibroblasts, 45% of the triglyceride is degraded within 4 hr after transfer to a medium containing either 10% fetal bovine serum or lipid-depleted serum (68). Likewise, bovine aortic endothelial cells enriched with either arachidonic or oleic acids lose 40% of the accumulated triglycerides in 6 hr (67).

IV. EFFECTS ON MEMBRANE PHYSICAL PROPERTIES

A. Fatty acid composition

Fatty acyl modifications can influence the degree of ordering and motion in the hydrocarbon core of the lipid bilayer, a property that commonly is referred to as membrane fluidity. Initially it was observed that changes in membrane fatty acid composition affected the transition temperature of concanavalin A binding and agglutination in LM cells (76), as well as agglutination of 3T3 cells induced by wheat germ agglutinin (14). These effects were obtained in intact cells. When the fatty acid composition of Ehrlich ascites cells was modified, electron spin resonance (ESR) measurements made in isolated membranes demonstrated changes in the order parameter (S) and the transition temperature of the probe motion parameter (τ_0) (38). The ESR probe used was 12-nitroxide stearic acid (12-NS), and plasma membranes were isolated from the Ehrlich cell homogenate before this probe was inserted. Similar ESR results were obtained with plasma membranes prepared from L1210 leukemic lymphoblasts having modified fatty acid compositions (40). The probes

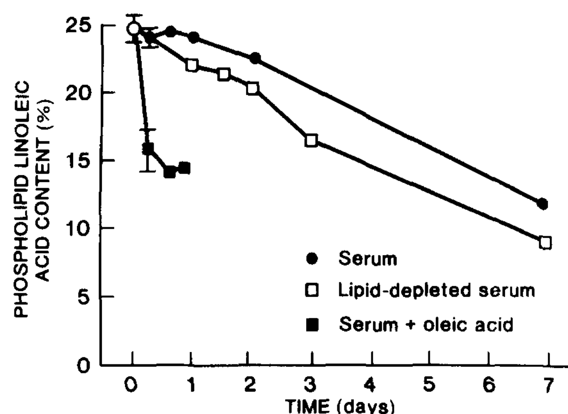

Fig. 2. Reversion of phospholipid fatty acyl modifications in cultured human skin fibroblasts. All cultures were enriched initially with linoleic acid. After washing, the cultures were transferred to one of three different maintenance media. These media contained a supplement of 10% fetal bovine serum, 10% lipid-depleted fetal bovine serum, or 10% bovine serum to which 75 μM oleic acid was added. Cultures were removed at various times for analysis of the phospholipid fatty acyl composition by gas-liquid chromatography. For simplicity, the only value shown is the linoleic acid content, given as a percentage of the total phospholipid fatty acyl groups.

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used in this study were 12-NS and 5-nitroxide stearic acid (5-NS). In both the Ehrlich ascites and L1210 cell plasma membranes, the changes in S and τ_0 associated with fatty acyl modifications are small. For example, the S values with 12-NS for Ehrlich ascites cell membranes enriched with 18:3, as compared with myristic acid (14:0), are 0.41 and 0.47, respectively (38). Fatty acid modification of Chinese hamster ovary cells produced differences in the temperature-dependence of fluorescence polarization in isolated plasma membrane preparations (59). This study was done with the fluorescent probe parinaric acid.

Four temperature transitions have been reported with spin label probes in plasma membrane preparations (77). Likewise, five transitions occur in LM cell membranes as measured by fluorescence with β-parinaric acid as the probe (78). However, the τ_0 results obtained with Ehrlich ascites and L1210 cell membranes were fitted adequately by assuming the presence of only two temperature transitions (38, 40). The higher τ_0 transition in Ehrlich ascites cell plasma membranes, which occurred at 31°C, was not altered appreciably by changes in phospholipid fatty acyl composition. By contrast, the lower transition increased from 20.5°C when the membrane was enriched with 18:3 to 25.0°C when it was enriched with 14:0 (38).

Changes in membrane fluidity also were observed when 5-NS was incorporated into intact L1210 cells (79). The plasma membrane component of the ESR spectrum was examined by allowing the signal to decay spontaneously during a 30-min incubation, and then reactivating that portion of the 5-NS located in the plasma membrane by exposure of the cells to 1 mM potassium ferricyanide (79).

An association between fatty acid compositional changes and membrane fluidity changes, however, does not occur in all systems. For example, no change in either S or the transition temperature of τ_0 occurred in plasma membrane preparations of EL4 murine T lymphocytes that were enriched with either saturated or unsaturated fatty acids (80). Since extensive fatty acyl modifications occurred in these plasma membranes (80), the T-lymphocyte must have a compensatory mechanism enabling it to resist changes in membrane fluidity.

B. Phospholipid composition

Studies with LM cells indicate that no change in membrane fluidity results from phospholipid head group modification. The head group composition was modified by growing the cells in culture media supplemented with choline, ethanolamine, N-methylethanolamine, or N,N-dimethylethanolamine (81, 82). Membrane viscosity ($\bar{\eta}$) was determined from fluorescence measurements. The probes that were used include DPH (81), parinaric acid (82), or 8-anilino-1-naphthalene sulfonic acid (82). In all cases negative results were obtained regarding phospholipid head group effects on $\bar{\eta}$. When the phospholipid head group composition is modified in LM cells, a concomitant change in fatty acyl chain length and degree of unsaturation occurs (83). This appears to be the compensatory mechanism that enables the LM cell to maintain membrane fluidity within narrow limits, a process termed homeoviscous adaptation (83).

C. Cholesterol content

The effect of changes in cholesterol content on plasma membrane fluidity have been studied extensively in the human erythrocyte. Fluorescence measurements with 12-(9-anthroyl) stearic acid as a probe indicate that increasing cholesterol content restricts molecular motion in the hydrophobic portion of the membrane lipid bilayer (84). Membrane $\bar{\eta}$ measured as the rotational diffusion of the fluorescence probe DPH also was affected by changes in cholesterol content (33). Increases in $\bar{\eta}$ occurred until the molar ratio of cholesterol to phospholipid reached 2.0, but not when the molar ratio was increased further.

V. EFFECTS ON CELLULAR FUNCTION

Many cellular functions and responses are affected when the membrane lipid composition is modified. The available information is only fragmentary, and there are a number of inconsistencies in the results. This makes it difficult to draw any general conclusions regarding functional responses to a given type of lipid modification.

A. Carrier-mediated transport

Table 4 lists the reported effects of membrane lipid modification on carrier-mediated transport in cultured cells. The initial observation was that enrichment of Ehrlich ascites cells with polyunsaturated fatty acid produced a 60% reduction in the K_m' for α-aminoisobutyrate uptake by the Na^+-dependent, high-affinity transport system (69). There was no change in the V_{max}' for α-aminoisobutyrate uptake. These measurements were made at 37°C. The activation energy of the Na^+-dependent transport component below its 35°C transition was 45% less than in corresponding cells enriched with saturated fat. Moreover, the lower transition temperature of the transport system was reduced from 19°C to 17°C when the cells were enriched in polyunsaturated fatty acids. Based upon these findings, it was concluded that the short-chain, neutral amino acid transport system operated more efficiently at physiological temperature when the cells were enriched with polyunsaturated fatty acid, presumably because its membrane lipid domain became more fluid. Similar results were obtained for the uptake of methotrexate, a folic acid analogue used in chemotherapy, by L1210 leukemic lymphoblasts enriched in

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TABLE 4. Effect of polyunsaturated fatty acid enrichment on carrier-mediated transport in cultured cells

Compound	Cell	Fatty Acid Enrichment	Effect on Transport Parameters	Reference
α-Aminoisobutyrate	Ehrlich ascites	Polyunsaturated	Decrease K'_m	69
	Y79 retinoblastoma	20:4, 22:6	No change	56
Methotrexate	L1210 leukemia	Polyunsaturated	Decrease K'_m	40
Melphalan	L1210 leukemia	Polyunsaturated	No change	86
Phenylalanine	Ehrlich ascites	Polyunsaturated	No change	85
Glutamate	Neuroblastoma M1	18:2, 18:3	Increase K'_m and V'_{max}	87
	Y79 retinoblastoma	20:4, 22:6	No change	56
Taurine	Neuroblastoma M1	18:2, 18:3	Increase K'_m and V'_{max}	87
	Y79 retinoblastoma	20:4, 22:6	Decrease K'_m	56
Choline	Y79 retinoblastoma	18:3, 20:4, 22:6	Decrease K'_m and V'_{max}	88
Glycine	Y79 retinoblastoma	18:2, 18:3, 20:4, 22:6	Increase K'_m and V'_{max}	58
Leucine	Y79 retinoblastoma	20:4, 22:6	No change	56
Serine	Y79 retinoblastoma	20:4, 22:6	No change	56

polyunsaturated fatty acid (40). In this case the K'_m was reduced by 40% at 37°C.

As opposed to these positive responses, no change in the kinetic parameters of phenylalanine uptake was observed when Ehrlich ascites cells were enriched with polyunsaturated fatty acid (85). The transition temperature of the phenylalanine transport system was lowered, however, as was noted for the α-aminoisobutyrate system. Likewise, the transition temperature for the transport of melphalan, a phenylalanine mustard used in chemotherapy, was lowered 24° to 20°C when L1210 cells were enriched with polyunsaturated fatty acid. However, the K'_m and V'_{max} for melphalan transport at 37° were not changed (86).

The kinetic properties of the glutamate and taurine transport systems in neuroblastoma M1 cells are sensitive to lipid modifications. In these cases, however, polyunsaturated fatty acid enrichment produced increases in K'_m and V'_{max} (87), an effect different from those noted for α-aminoisobutyrate and methotrexate transport in Ehrlich ascites and L1210 cells, respectively.

Enrichment of human Y79 retinoblastoma cells with polyunsaturated fatty acids affected the transport at 37°C of three substances, choline (88), glycine (58), and taurine (56). By contrast, the uptake of leucine, serine, glutamate, and α-aminoisobutyrate was unaffected (56). The negative results with glutamate and α-aminoisobutyrate differ from what has been observed in neuroblastoma (87) and Ehrlich cells (69), respectively. Moreover, the three compounds that were influenced by lipid modification did not all respond in the same way. Polyunsaturated fatty acid enrichment decreased the K'_m for choline and taurine high-affinity uptake (56, 88), whereas it increased the K'_m for glycine high-affinity uptake (58).

The differences in the responses of the α-aminoisobutyrate, glutamate, and taurine transport systems in different cells may be due to species variations, tissue differences, or the fact that the polyunsaturated fatty acids used for enrichment were not identical in all cases. Additional studies will be needed to determine which of these possibilities actually is responsible. The different results in Ehrlich cells with α-aminoisobutyrate and phenylalanine (69, 85), in L1210 cells with methotrexate and melphalan (40, 86), and in the Y79 retinoblastoma cells with choline and taurine as opposed to glycine, glutamate, leucine, serine, and α-aminoisobutyrate (56, 58, 88) indicate that all of the transport carriers contained in a cell do not respond uniformly to the same membrane lipid modifications. Some are completely unresponsive with respect to their kinetic properties, others are modulated so that they are able to mediate transport more efficiently, and still others are modulated so that their transport efficiency is reduced. One possibility is that the membrane lipid environment fine-tunes only those transporters that must operate within narrow limits in a particular cell. Those that take up substances especially vital for cellular function are positively modulated; others that take up substances which should remain extracellular are negatively modulated.

The mechanism whereby lipids can influence the properties of certain transporters is presently unknown. A likely possibility is that the surrounding lipids affect the conformation of certain carriers, thereby enhancing or reducing the accessibility of their binding sites. If so, then the conformations of only certain transporters are sensitive to lipids, probably because only in these cases is the structure of the protein segment that passes through the lipid bilayer such that it can be affected by lipid modifications. Alternatively, the lipid microenvironments around various transporters may differ, with only those transporters located in microenvironments that are changed by a particular type of lipid modification being affected. In two of the cases where the kinetic parameters did not change in response to phospholipid fatty acyl modifica-

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tions, phenylalanine transport in Ehrlich cells (85) and melphalan transport in L1210 cells (86), the transition temperatures of the transport systems still were affected. Therefore, these two systems actually are lipid-sensitive even though this sensitivity is not transmitted to the operation of the carrier under ordinary conditions. The transition temperatures of other systems that do not exhibit kinetic changes have not been measured. Based on the available evidence, however, it seems reasonable to suggest that all transmembrane carriers may be lipid-sensitive, but that only in certain cases are the interactions critical enough to affect transport.

B. Membrane-bound enzymes

The activity and properties of a number of membrane-bound enzymes have been examined following lipid modifications in cultured cells. These enzymes are listed in **Table 5**. Adenylate cyclase activity is affected by changes in the composition of the phospholipid fatty acyl chains and polar head groups, as well as by changes in the cholesterol content of the plasma membrane. In LM cells grown in a medium containing choline, enrichment with 18:2 increases the prostaglandin E_1 (PGE_1)-stimulated activity (89). When the cells were enriched with both 18:2 and ethanolamine, the basal activity was increased but the PGE_1-stimulated activity was decreased. Changes in the membrane phospholipid head group composition also increased the basal, NaF- and thyrotropin-stimulated adenylate cyclase activity in human skin fibroblasts (90). To explain these effects, it has been suggested that adenylate cyclase can exist in several different conformations having varying activities, and that changes in membrane lipid composition cause the enzyme to shift from one conformation to another (45).

Elevations in the membrane cholesterol content in-

TABLE 5. Effects of lipid compositional changes on membrane-bound enzymes in cultured cells

Enzyme	Cell	Lipid Modification	Effect	Reference
Adenylate cyclase	LM	18:2	Basal and PGE_1-stimulated activity changed	89
	Human skin fibroblasts	Phospholipid head groups	Increased basal, NaF-, and thyrotropin-stimulated activity	90
	CHO	Cholesterol	Basal, PGE_1-, and NaF-stimulated activities changed	91
	Kidney fibroblasts	Cholesterol and dipalmitoyl phosphatidylcholine	Decreased activity	92
	Platelets	Cholesterol	Basal, PGE_1-, and NaF-stimulated activity changed	93
$Na^+ + K^+$-ATPase	Ehrlich ascites	Polyunsaturated fatty acid	Transition temperature, activation energy	94
	Human skin fibroblasts	Phospholipid head groups	No change	24, 90
	Kidney fibroblasts	Cholesterol and dipalmitoyl phosphatidylcholine	Decreased activity	92
	Lymphocytes	18:2, 20:4	Increased activity	95
Lysolecithin acyltransferase	Lymphocytes	18:2, 20:4	Increased activity	95
Mg^{2+}-ATPase	Lymphocytes	18:2, 20:4	Decreased activity	95
γ-Glutamyl transferase	Lymphocytes	18:2, 20:4	Decreased activity	95
	Human skin fibroblasts	Phospholipid head groups	No change	90
p-Nitrophenyl phosphatase (alkaline)	Lymphocytes	18:2, 20:4	Decreased activity	95
5'-Nucleotidase	Human skin fibroblasts	Phospholipid head groups	No change	24, 90
Acylcoenzyme A:cholesterol transferase	Ehrlich ascites	Polyunsaturated fatty acid	Decreased activity	96
NADPH-cytochrome c reductase	LM	Phospholipid head groups	No change	24
Glucose 6-phosphatase	LM	Phospholipid head groups	No change	24
Inosine diphosphatase	LM	Phospholipid head groups	No change	24
Succinate cytochrome c reductase	LM	Phospholipid head groups	No change	24

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crease basal adenylate cyclase activity in Chinese hamster ovary cells, but the PGE_1- and NaF-stimulated activities are decreased (91). In rat kidney fibroblasts, supplementation with cholesterol and dipalmitoyl phosphatidylcholine decreases adenylate cyclase activity (92). Adenylate cyclase activity also is modified in human platelets by modification in the membrane cholesterol content (93). When the cholesterol content is increased, the basal activity of adenylate cyclase also increases, but the enzyme is no longer stimulated by either PGE_1 or NaF. A decrease in the platelet cholesterol content reduces basal adenylate cyclase activity. These changes are thought to result from physical effects of cholesterol on the platelet membrane phospholipids that interact with the enzyme.

Changes in the fatty acyl composition and cholesterol content of the plasma membrane also can affect $Na^+ + K^+$-ATPase. In Ehrlich cells, increases in polyunsaturated fatty acid change the transition temperature and lower the activation energy of the enzyme below this transition (94). There was no change in $Na^+ + K^+$-ATPase activity in human skin fibroblasts when the phospholipid head group composition was modified (24, 90), but increases in cholesterol content produce a decrease in $Na^+ + K^+$-ATPase activity in rat kidney fibroblasts (92). Increases in the 18:2 or 20:4 content of the plasma membrane phospholipids in calf thymus lymphocytes increase the $Na^+ + K^+$-ATPase activity (95). Enrichments with 18:2 and 20:4 also produce an increase in lysolecithin acyltransferase activity and decreases in the activities of Mg^{2+}-ATPase, γ-glutamyl transferase, and alkaline *p*-nitrophenyl phosphatase (95). Smaller but similar effects were produced by enrichment with 18:1, whereas enrichment with 16:0 had no effect. Phospholipid head group modification of human skin fibroblasts had no effect on either γ-glutamyl transferase activity (90) or 5'-nucleotidase activity (24, 90).

An increase in polyunsaturated fatty acids, primarily 18:2, reduced the acylcoenzyme A:cholesterol acyltransferase activity in Ehrlich ascites cell microsomes (96). This is due to a reduction in K'_m for the acylcoenzyme A substrate. The generality of this effect is questionable, however, because the opposite response occurs in rat liver microsomes where an increase in polyunsaturated fatty acids leads to an increase in acylcoenzyme A:cholesterol acyltransferase activity (97, 98). A possible explanation for the difference is that in the liver microsomes, there is a large increase in 20:4 in addition to the elevated 18:2 content when they become enriched with polyunsaturated fatty acid (97).

Acylcoenzyme A:cholesterol acyltransferase of Ehrlich cell microsomes can be solubilized with detergent and reconstituted in liposomes (99). The activity of the solubilized preparation depends on the fatty acid composition of the liposomes, the highest activity occurring with dioleoyl phosphatidylcholine and the lowest with totally saturated phosphatidylcholines. The activity of acylcoenzyme A:cholesterol acyltransferase of rat liver microsomes also can be modulated by incubation with liposomes in the presence of phospholipid exchange protein (100). This produces changes in the microsomal phospholipid fatty acyl composition. Again, the highest activities occur following enrichment with dioleoyl phosphatidylcholine, the lowest with dipalmitoyl phosphatidylcholine. Therefore, both of these methods for modifying the membrane phospholipid fatty acyl composition lead to the same overall conclusion as the data obtained from modified Ehrlich cells; acylcoenzyme A:cholesterol acyltransferase activity is sensitive to the composition of the surrounding membrane lipids. The mechanism of this effect could be differences in the accessibility of membrane cholesterol to the enzyme rather than a physical effect of the membrane phospholipids on the enzyme.

There were no changes in the activities of several membrane-bound enzymes when the phospholipid head groups of LM cells were modified (24). These include NADPH-cytochrome c reductase, glucose 6-phosphatase, inosine diphosphatase, and succinate cytochrome c reductase. In this work the phospholipids were enriched with ethanolamine, N-methylethanolamine, or N,N-dimethylethanolamine. Thus, except in the case of adenylate cyclase (90), the available evidence suggests that the nonpolar components of the membrane lipid bilayer, the phospholipid fatty acyl chains and cholesterol, exert a greater effect than phospholipid head groups in modulating the activity of membrane-bound enzymes.

C. Receptors

Table 6 lists the effects of lipid modification on the properties of membrane receptors in cultured cells. An increase in the unsaturated fatty acid content of the membrane phospholipids affects the binding properties of the insulin receptor in Friend erythroleukemia and Ehrlich ascites cells (101, 102). In both cases the cells enriched in unsaturated fatty acids contained an increased number of insulin receptors and exhibited a decrease in receptor binding affinity. The receptor number more than doubled when the cells were enriched with 18:2. This was associated with a decrease in the affinity of both the unoccupied ($\bar{K}_e$) and occupied ($\bar{K}_f$) forms of the receptor. By contrast, bovine pulmonary artery and aortic endothelial cells show no changes in the binding of either insulin or multiplication stimulating activity, an insulin-like growth factor, in spite of extensive phospholipid fatty acyl modifications (103). Likewise, these changes produced little effect on either the insulin induced down-regulation of the receptor or in the cellular processing of insulin. Therefore, it is questionable whether the positive responses observed in the Friend erythroleukemia and Ehrlich ascites cells are generalized effects. Yet, solubilization of the insulin receptor from turkey erythrocyte membranes,

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TABLE 6. Effect of lipid modifications on the properties of receptors in cultured cells

Receptor	Cell	Lipid Modification	Effect	Reference
Insulin	Friend erythroleukemia	18:1, 18:2	Changes in n, $\bar{K}_e$, $\bar{K}_f$	101
	Ehrlich ascites	Polyunsaturated fatty acid	Changes in n, $\bar{K}_e$, $\bar{K}_f$	102
	Bovine aortic and pulmonary artery endothelium	16:0, 18:1, 18:2, 20:4	No change in binding	103
Multiplication stimulating activity (MSA)	Bovine aortic and pulmonary artery endothelium	16:0, 18:1, 18:2, 20:4	No change in binding	103
Opiate	Neuroblastoma X glioma	Polyunsaturated fatty acid	Decreased binding	106
α-Adrenergic	Human platelets	Cholesterol	No change in binding	107
Acetylcholine	Chick embryo myotubes	18:1, 18:1 *t*	No substantial change in lateral mobility	108

followed by its reconstitution in liposomes of different fatty acyl composition, produced effects similar to those seen in the Friend erythroleukemia and Ehrlich ascites cells. Reconstitution in more unsaturated liposomes increased the insulin binding capacity and reduced the affinity of the receptor (104). Changes in the fatty acyl composition of phosphatidylcholine liposomes also influenced the binding of thyrotropin to the solubilized, reconstituted glycoprotein component of the thyrotropin receptor (105).

Binding to the opiate receptor is affected by changes in cellular fatty acid composition. The binding of labeled enkephalin to neuroblastoma X glioma cell membranes enriched with polyunsaturated fatty acid was decreased, with no change in receptor affinity (106). The binding of labeled dihydromorphine and naloxone was similarly decreased. Thus, the response to polyunsaturated fatty acid enrichment is opposite from that noted with the insulin receptor, where the binding affinity decreases but the number of available binding sites increases (101, 102).

As opposed to these positive responses, enrichment of platelet membranes with cholesterol produced no change in the number of α-adrenergic receptors or in the binding of dihydroergocryptine or epinephrine to these receptors (107). In addition, enrichment of cultured chick embryo myotubes with either 18:1 or elaidic acid, the *trans*-isomer of 18:1, did not appreciably affect the lateral mobility of the acetylcholine receptor (108).

D. Phagocytosis, endocytosis and exocytosis

In mouse peritoneal macrophages, an increase in unsaturation produces an enhancement in phagocytosis, the ingestion of *Shigella flexneri* being increased twofold (109). Likewise, enrichment with 18:1 as compared with 16:0 produces a three- to fourfold increase in erythrophagocytic activity (110). These fatty acid modifications do not influence the ability of the macrophages to kill intracellular bacteria or to generate superoxide anions after stimulation with phorbol myristate acetate or opsonized zymosan (110). A reduction in endocytosis also occurs in peritoneal macrophages that are enriched in saturated fatty acids (52, 111).

The effect of lipid modification on depolarization-dependent exocytosis has been examined in two neural cell lines. Enrichment with unsaturated fatty acid decreased the release of norepinephrine from PC12 pheochromocytoma cells following stimulation of the nicotinic cholinergic receptors with carbamylcholine (55). The same response was observed when the cells were stimulated with veratridine. By contrast, unsaturated fatty acid enrichment had no effect on the K^+-stimulated release of epinephrine (55). Y79 retinoblastoma cells rapidly release glycine when they are depolarized with K^+ (112). As in the case of the pheochromocytoma cells, fatty acid modification did not appreciably affect K^+-dependent glycine release (Yorek, M. A., and A. A. Spector, unpublished observations). Thus, the effect of lipid modification is more likely to involve either the cholinergic receptor or sodium channels rather than the exocytosis process itself.

E. Cytotoxicity

Rat hepatoma cells enriched with either 18:1 or 18:2 are more susceptible to complement-dependent cytolysis and spleen cell-mediated cytotoxicity (51, 113). Enrichment with 18:2 was twice as effective as 18:1 in stimulating complement-mediated cytolysis, and the cells enriched with 18:2 showed a higher initial rate of cytolysis (51). Although both 18:1 and 18:2 stimulated natural killer cell cytotoxicity equally after 2 days in culture, 18:2 enrichment became more effective after longer periods (113). However, these effects of fatty acid modification were not exhibited by all cells. For example, EL-4 cells showed the expected change in plasma membrane fluidity when various fatty acids were added to the culture medium, but there was no effect on cytolysis produced by either antibody and complement or effector cells (114, 115).

Changes in the cytotoxicity produced by the chemotherapeutic drug adriamycin occur when L1210 leukemic

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lymphoblasts are enriched with certain fatty acids (116). Enrichment with 22:6 increased the cytotoxic effect as compared with cells grown in either unsupplemented medium or with an 18:1 supplement. The sensitivity to adriamycin increased as the 22:6 content of the cell phospholipids increased.

L1210 leukemic lymphoblasts enriched with 22:6 are more thermosensitive than corresponding control cultures or those enriched with 18:1 (117). Thermal sensitivity increased as the 22:6 content of the L1210 cell membranes was raised, and differential effects were apparent at temperatures between 41°C and 44°C. P388 macrophages also became more thermosensitive when they were enriched with polyunsaturated fatty acids (118).

F. Prostaglandin production

Modification of the phospholipid fatty acyl composition can affect prostaglandin production by cultured cells. Enrichment with 18:2 in MDCK and 3T3 cells increases the production of PGE_2, the main prostaglandin formed by these cultures (43, 46). MDCK and 3T3 cells convert 18:2 to 20:4, and the 20:4 content of the cell phospholipids increases. The greater PGE_2 formation apparently is due to the increased availability of 20:4 in the intracellular phospholipid storage pools that provide the substrate for prostaglandin synthesis. Even in control cultures, however, far more 20:4 is present in cellular phospholipids than is needed to support maximal prostaglandin production. Therefore, why should a further enrichment with 20:4 increase prostaglandin output? The most likely possibility is that the intracellular pools that release 20:4 in response to stimuli are relatively small and have a rapid turnover, such as 1-alkyl-2-acyl-*sn*-glycerol-3-phosphocholine (119), ethanolamine plasmalogen (120), or phosphatidylinositol-4,5-bisphosphate (121–123). Because these pools are very labile, their 20:4 content may vary considerably and be susceptible to rapid fluctuations even when the total phospholipids have what appears to be an excess of 20:4.

The system that has been studied most extensively with regard to prostaglandin production is endothelial cells. **Fig. 3** shows the effect of different fatty acid enrichments on the capacity of the endothelial cells to produce prostacyclin (PGI_2). These cultures were stimulated with thrombin, so that the 20:4 needed for PGI_2 production is derived from the intracellular storage pools. The largest effects were observed with 18:2, 20:4, and 20:5, but some reduction in PGI_2 output also occurred following enrichment with 18:1 and 22:6.

When endothelial cells are enriched with 18:2, they produce about 70% less PGI_2 in response to thrombin. Likewise, less PGI_2 is produced when the cells are stimulated with the calcium ionophore A23187 (42, 43). Inhibition was not produced when the cells were enriched with linolelaidic acid, the *trans*-isomer of 18:2. Platelets also produce less thromboxane A_2 (TXA_2) when they are enriched in 18:2 (48). Endothelial cells and platelets do not convert much 18:2 to 20:4 under ordinary culture conditions (41, 51), and the 20:4 content of their phospholipids decreases when they become highly enriched in 18:2 (42, 43, 48). The decrease in prostaglandin output is due in part to the reduction in the 20:4 content of the intracellular phospholipid storage pools. This probably also explains the decrease in PGI_2 production when the endothelial cells are enriched with 18:1, for the 20:4 content of the cell phospholipids is reduced in this case by about 15 to 25% (42). Another likely cause of the decrease in PGI_2 output when the cells are enriched with 18:2 is that either 18:2 itself or 20:2, an elongation product of 18:2, inhibits the cyclooxygenase reaction when they accumulate intracellularly (42). When low concentrations of 20:4 were added to the incubation medium as substrate, PGI_2 production by the cells enriched with 18:2 remained suppressed. As the concentration of 20:4 was raised, the suppression was gradually overcome (42). This suggests that the inhibition of PGI_2 production by 18:2 enrichment is competitive and that it can be overcome if an excess of 20:4 is available to the cyclooxygenase.

Enrichment of 3T3 and 3T3-L1 cells with 20:4 itself increased PGE_2 production when the cultures were subsequently stimulated with the calcium ionophore A23187 (46, 124). No appreciable increase in PGE_2 production was observed, however, when MDCK cells were enriched with 20:4 under similar conditions (50). Furthermore, as

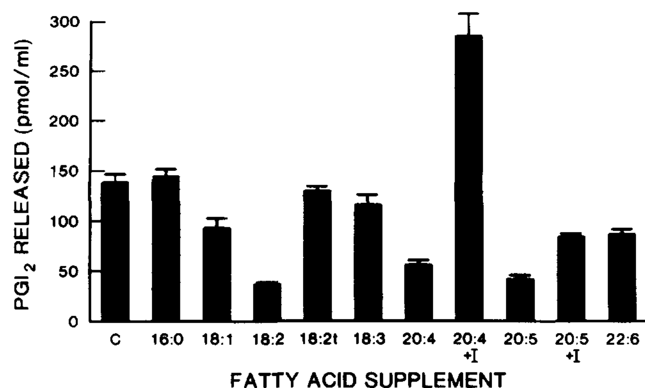

Fig. 3. Effect of enrichment of cultured human umbilical vein endothelial cells on PGI_2 production. Endothelial cultures were incubated for 18 hr with media containing 10% fetal bovine serum and 100 μM supplemental fatty acid. These media were removed, and the cultures were washed with a solution containing fatty acid-poor albumin. The cultures were then stimulated for 5 min with a medium containing 1 U of thrombin, and the PGI_2 release was measured by radioimmunoassay for 6-keto-$PGF_{1\alpha}$. With 20:4 and 20:5, some cultures were enriched in the presence of 100 μM ibuprofen, which is abbreviated as I. The abbreviation C refers to cultures that were incubated in a medium without any supplemental fatty acid. Each bar represents the mean ± SE of values obtained from four separate cultures.

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shown in Fig. 3, enrichment of endothelial cells with 20:4 considerably inhibited PGI_2 release when the cells were stimulated subsequently with thrombin (125). If the endothelial cultures are enriched with 20:4 in the presence of ibuprofen, a reversible cyclooxygenase inhibitor, this inhibition was prevented and, as seen in Fig. 3, the subsequent release of PGI_2 in response to thrombin was twice as great as in control cultures (125).

These results can be explained by the fact that 20:4 enrichment of endothelial cells produces a concomitant inhibition of the cyclooxygenase reaction. This probably is due to the large quantity of prostaglandins that is produced during the period of exposure of 20:4 (125), for there is evidence that the cyclooxygenase self-deactivates in some systems when it is highly active (126). If ibuprofen is present, prostaglandins are not produced during the period of enrichment with 20:4, and the cyclooxygenase remains active and is able to respond when the ibuprofen is removed and the cells are subsequently stimulated with thrombin. Since the phospholipid precursors pools are enriched with 20:4 under these conditions (125), the cells respond to the subsequent stimulation with a greater PGI_2 output. For unexplained reasons, the cyclooxygenase of 3T3 and 3T3-L1 cells does not deactivate as a result of exposure to similar concentrations of 20:4, even though these cells also produce large quantities of prostaglandins during the enrichment period.

As shown in Fig. 3, enrichment of endothelial cultures with 20:5 reduces the PGI_2 output when the cells are stimulated subsequently with thrombin (65). Likewise, enrichment with 20:5 inhibits thromboxane TXA_2 synthesis in platelets (127), PGI_2 synthesis in smooth muscle cells (128, 129), rat glomerular epithelial cells (129), and MDCK cells (129), and both PGE_2 and $PGF_{2\alpha}$ synthesis in rat glomerular epithelial cells, MC5-5 mouse fibroblasts, $HSDM_1C_1$ mouse fibrosarcoma cells, MDCK cells, 3T3 cells, and J-111 monocytic leukemia cells (129). Fig. 3 also shows that enrichment of endothelial cells with 20:5 in the presence of ibuprofen is only partially protective. Under these conditions, the PGI_2 output is still 40% below the control value. This suggests that some self-deactivation of the cyclooxygenase may occur as a result of 20:5 enrichment of the endothelial cells, perhaps because of the small amount of PGI_3 that is formed (49). With regard to the latter, 20:5 is converted by endothelial cells to a PGI_3-like product, but only at a rate of about 8% that of 20:4 conversion (65). However, this can account for only a part of the total reduction in PGI_2 output. The remainder of the inhibition apparently is due to two additional processes, reduction in the 20:4 content of the intracellular phospholipid storage pools and competition between 20:4 and 20:5 for the cyclooxygenase (65). No studies have as yet been done to determine why enrichment with 22:6 reduces endothelial PGI_2 production.

As opposed to prostaglandin production, enrichment of cells with 20:5 does not inhibit the total output of lipoxygenase products. In some cases the output of hydroxyeicosatetraenoic acids actually is increased when the 20:5 content is elevated (129).

All of these findings can be explained by a mechanism involving changes in the polyunsaturated fatty acid composition of the intracellular substrate storage pools. One observation made with both MDCK and 3T3 cells (46, 50), however, cannot be readily explained by such a mechanism. This occurred when the MDCK cells were enriched with 18:2, or the 3T3 cells were enriched with either 18:2 or 18:1. PGE_2 production by these modified cells was elicited by adding arachidonic acid to the extracellular fluid. Under these conditions, the PGE_2 formed is derived almost entirely from the external arachidonic acid. Yet, PGE_2 production was increased when the cells were enriched with these unsaturated fatty acids (46, 50). This suggests that other factors besides changes in the fatty acid composition of the intracellular storage pools may play a role in the prostaglandin effects; for example, a change in the fluidity of the membranes in which the prostaglandin-forming enzymes are embedded.

G. Cell growth

Under certain conditions fatty acid supplementation can affect cell growth. When LM cells are grown as a suspension in a serum-free, chemically defined medium, supplementation with 100 μM palmitic acid inhibits growth (130). No effects on growth occurred with unsaturated fatty acid supplements, and the inhibitory effects of palmitic acid were overcome if unsaturated fatty acids also were added to the medium. A good correlation was found between the unsaturated fatty acid content of the membrane phospholipids and growth, and inhibition always occurred when the total percentage of unsaturated fatty acids in the LM cell phospholipids decreased to less than 50%.

Enrichment with palmitic acid at concentrations above 50 μM also inhibited the growth of human skin fibroblasts (15). As in the case of LM cells, no effects on growth occurred with supplements of either oleic or linoleic acid. A small amount of inhibition occurred with linolenic acid (15, 60), and arachidonic acid reduced growth by 25 to 50% at concentrations between 50 and 100 μM (15). Eicosapentaenoic acid at a concentration of 100 μM reduced the growth of both human skin fibroblasts and Y79 retinoblastoma cells by 50%, and docosahexaenoic acid reduced the growth of human skin fibroblasts by 25% at concentrations between 30 and 100 μM (60). It is likely that growth is inhibited when the cells are enriched with these polyunsaturates because of an excessive formation of lipid peroxides (131, 132).

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VI. MECHANISM OF LIPID DEPENDENCE

A. Membrane lipid structure

Most of these effects on cellular function probably are caused directly by the modifications in membrane lipid composition. According to this view, certain receptors, transporters, and enzymes are sensitive to the structure and physical properties of the lipids with which they interact. This sensitivity may involve conformational changes that affect the binding sites of receptors and transporters or the active site of enzymes. Other properties that may be affected include the lateral mobility of these proteins within the lipid bilayer or their interactions with other membrane components. Any of these perturbations could produce a functional change. Spin label and fluorescence measurements indicate that the lipid modifications possible in biological systems are sufficient to have an effect on membrane fluidity (38, 40, 59, 77, 79, 81, 82, 84). It seems reasonable to assume that if the lipid microenvironment sensed by a probe is altered, a protein embedded within this lipid domain may also sense the difference and thereby operate in a somewhat different manner. Thus, the membrane lipid structure mechanism may involve either bulk lipid fluidity, localized changes in specific lipid domains (133), or a combination of both.

Perhaps the strongest evidence in favor of a causal relationship between lipid structure and membrane function comes from studies with the insulin receptor. Changes in insulin binding similar to those produced by enriching certain cells with polyunsaturated fatty acids (101, 102) occur when unmodified receptors are solubilized and reconstituted in liposomes having a greater amount of unsaturation (104). In the case of prostaglandin formation, the phospholipid fatty acyl modifications probably exert their effect primarily through a change in substrate availability. The chemical basis of the mechanism is the same, however, a change in membrane lipid composition.

Much of the evidence linking changes in membrane lipid composition with altered function is based on correlations, and it is difficult to obtain conclusive proof of cause and effect. Since the methods used to modify membrane lipids may produce additional perturbations, the possibility that other mechanisms may be responsible for certain of the functional alterations must be considered.

B. Fatty acids and triglycerides

Alterations in cellular function can be produced by free fatty acids (134). These include effects on transport such as the Na^+-dependent uptake of γ-aminobutyrate, proline, aspartate, and glutamate in brain slices and synaptosomes (135–138). It has been proposed that free fatty acids cause these effects by altering $Na^+ + K^+$-ATPase activity, Ca^{2+} movement, or guanylate cyclase activity (138–140). A small amount of free fatty acid may be generated as a result of the continuous turnover of membrane phospholipid fatty acyl groups (8, 71, 141–144). It is possible that when the phospholipid fatty acyl composition is modified, the composition of the free fatty acid that is generated may be altered, and processes sensitive to fatty acid composition could be affected. Thus, the possibility that free fatty acids may be the direct mediators of some of the functional effects must be considered.

High concentrations of supplemental fatty acid also cause intracellular triglyceride accumulation, often in the form of cytoplasmic lipid inclusions (8, 67, 145, 146). When the cultures are transferred to an unsupplemented medium, as is often done for function studies, the triglycerides undergo hydrolysis (67, 68). Either the lipid accumulations or the hydrolytic products might influence cellular processes, and this should be considered as a possible cause for some of the functional changes. Furthermore, the increase in lipid peroxidation that can result from enrichment with polyunsaturated fatty acid may have effects on other cellular processes besides inhibiting cell proliferation (131, 132).

C. Acylation of membrane proteins

A number of membrane glycoproteins contain covalently bound long-chain fatty acid in ester linkage (147). These include the transferrin receptor (148, 149), myelin proteolipid protein (150–152), and rhodopsin (153). In addition, amide-linked acylation occurs in $pp60^{src}$ (154), the α and β subunits of the nicotinic cholinergic receptor (155), and the cyclic AMP-dependent protein kinase (156). Since not all membrane proteins are acylated, the process must have a specific function such as facilitating the attachment of the protein to the lipid bilayer, channeling proteins for recycling, or inducing a conformational change (157).

Only saturated fatty acids have been tested so far as acylating agents in intact cells. However, about 5% of the palmitic acid that is covalently incorporated into myelin proteolipid protein is converted to 18:1 (151, 152). Likewise, oleic acid can be covalently incorporated into viral polypeptides that are acylated by microsomal membrane preparations (158). A small amount of covalently bound 18:2 also appears to be present in the myelin proteolipid protein (152), and there seems to be no reason a priori why polyunsaturates should not be acylated. If so, they may replace covalently bound saturated fatty acid if the cell becomes sufficiently enriched with polyunsaturates. Such a mechanism also should be considered as a possible cause for some of the functional changes that are associated with polyunsaturated fatty acid enrichment.

VII. PHYSIOLOGIC RELEVANCE

It is now clear that variations can occur in the mem-

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brane lipids of intact, viable mammalian cells. These changes can be of sufficient magnitude to alter membrane physical properties and affect certain membrane functions. Thus, the basic questions regarding applicability to living systems have been answered during the last decade. Moreover, the same types of membrane fatty acid compositional variations that can be produced in cultured cells occur in animals when the dietary fat intake is modified (97, 98, 159). This is associated with changes in a number of membrane functions in the animal, including the activity of membrane bound enzymes (97, 98), neurotransmitter uptake in the brain (160), β-adrenergic receptor binding properties (161), and prostaglandin synthesis (162-164). Membrane fatty acid alterations also have been produced in vivo in human erythrocytes and platelets by dietary fat modifications (165-167). The most striking functional effects in humans have resulted from eicosapentaenoic acid enrichment, which causes changes in prostaglandin formation, platelet aggregation, and blood coagulation (166-170). Therefore, not only are membrane fatty acid compositional changes possible in humans under physiologic conditions, they may provide new approaches for the prevention and treatment of certain diseases. This provides added impetus to continue basic studies designed to determine more about the underlying molecular mechanisms and their functional implications.

VIII. PRESENT STATUS AND FUTURE DIRECTIONS

It is not yet possible to classify the effects of lipid composition on membrane function according to a consistent pattern. The available information is too fragmentary, and there is too much diversity in the existing data to permit generalizations. Cell culture appears to be the best approach for pursuing this problem. A much wider array of modifications is possible in cultured cells than in intact animals, and the conditions can be controlled better. Through this approach, more complete data regarding lipid effects on membrane enzymes, receptors, transport systems, and energy transducing systems should be obtained in a variety of cell types. Cell culture also lends itself to the exploration of important unresolved questions, such as the functional effects of the n-3 polyunsaturated fatty acids (64), the eicosatrienoic acid that accumulates in essential fatty acid deficiency (41, 66), the hydroxyeicosatetraenoic acids formed by the lipoxygenase pathways (171), and the phospholipid ethers (172). Since sphingolipids can be taken up and incorporated into membranes, this approach can be employed to learn more about their role in cellular function. Finally, the experimental designs used for the prostaglandin studies can be applied to determine whether similar lipid modifications also affect leukotriene production.

Cultured cell systems are too complex, however, to provide conclusive evidence regarding mechanism. Even isolated membrane preparations are too complicated for this purpose, especially if the lipid modifications are produced while the cell or organism is still intact. An approach better suited to mechanistic interpretations is solubilization of membrane components and their reconstitution in phospholipid vesicles (104). Another good approach for this purpose is to use membrane vesicles in which the lipid composition is modified by incubation with liposomes and a phospholipid exchange protein (100). When these systems are used alone, serious questions often can be raised regarding physiologic relevance. Therefore, we believe that the best approach is to make functional observations with cultured cells and then explore the mechanism producing these effects with either a reconstituted system or isolated membranes modified by incubation with liposomes.

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