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MODIFICATION OF THE FATTY ACID COMPOSITION OF EHRLICH ASCITES TUMOR CELL PLASMA MEMBRANES

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SUMMARY

The fatty acyl group composition of Ehrlich ascites tumor cell plasma membranes was modified by feeding the tumor-bearing mice diets rich in either coconut or sunflower oil. When coconut oil was fed, the oleate content of the membrane phospholipids was elevated and the linoleate content reduced. The opposite occurred when sunflower oil was fed. Qualitatively similar changes were observed in the plasma membrane phosphatidylethanolamine, phosphatidylcholine and mixed phosphatidylserine plus phosphatidylinositol fractions. These diets also produced differences in the sphingomyelin fraction, particularly in the palmitic and nervonic acid contents. Unexpectedly, the saturated fatty acid content of the plasma membrane phospholipids was somewhat greater when the highly polyunsaturated sunflower oil was fed. The small quantities of neutral lipids contained in the plasma membrane exhibited changes in acyl group composition similar to those observed in the phospholipids. These fatty acyl group changes were not accompanied by any alteration in the cholesterol or phospholipid contents of the plasma membranes. Therefore, the lipid alterations produced in this experimental model system are confined to the membrane acyl groups.

INTRODUCTION

One method for investigating the role of lipids in membrane function is to alter the fatty acyl group composition of the membrane phospholipids. This has been accomplished in microorganisms [1–5] and mammalian cells [6–10] in vitro by modifying the fatty acid content of the culture fluid. It also has been achieved in erythrocytes [11, 12] and hepatocytes [13, 14] in vivo by modifying the lipid composition of the diet. These observations led us to think that it might be possible to produce similar modifications in cancer cells.

Working with the Ehrlich ascites tumor, we observed that the fatty acid composition of the cells could be altered appreciably by feeding the tumor-bearing mice diets containing different kinds of fats [15]. We thought that this system would be ideal for plasma membrane studies for three reasons. First, crude membrane fractions that we isolated from Ehrlich cell homogenates between 10 000 and 100 000 $\times g$ exhibited changes in fatty acid composition similar to those found in the intact homo-

genate [15]. This suggested that the dietary fat manipulations that we employed would alter membrane fatty acid composition. Second, methods for preparing quite pure plasma membranes from Ehrlich ascites cells already are available [16–18]. Finally, very large quantities of Ehrlich cells can be grown routinely, making the preparation of enough plasma membranes for extensive enzymatic or transport studies feasible. Because of the potential usefulness of this experimental system, we have examined in detail the variations in plasma membrane lipid composition that occur when the tumor-bearing mice are fed a diet rich in either saturated or polyunsaturated fats.

MATERIALS AND METHODS

Animals and diets

Weanling male CBA mice were fed a semisynthetic diet containing 26 % casein, 10 % corn starch, 43 % sucrose, 4 % mineral mix and 1 % vitamin mix (Teklad, Madison, Wisconsin) supplemented with either 16 % sunflower oil or 16 % coconut oil [15, 19]. The fatty acid compositions of these diets are presented in Table I. Mice were fed these experimental diets for 4 weeks prior to intraperitoneal implantation of $4 \cdot 10^6$ Ehrlich cells. The experimental diets were continued during tumor growth, and the tumors were harvested 13 days after transplantation.

TABLE I

FATTY ACID COMPOSITION OF SUNFLOWER AND COCONUT OIL DIETS

Fatty acid*	Composition (%)	
	Sunflower	Coconut
8 : 0	–	5.0**
10 : 0	–	6.6
12 : 0	–	52.9
14 : 0	–	18.7
16 : 0	6.0	7.9
18 : 0	4.8	2.1
18 : 1	15.6	5.4
18 : 2	70.8	1.4
18 : 2***	2.8	–

* Chain length: number of double bonds.
** Values are the averages of 2 assays.
*** Fatty acids with retention times longer than 18 : 2.

Plasma membrane isolation

Cells obtained from 3–5 mice were pooled, separated from the ascites plasma by centrifugation and washed [20]. A cell homogenate was prepared by nitrogen cavitation and plasma membranes were isolated from the crude microsomal fraction of this homogenate by the method of Molnar et al. [17]. The purity of the plasma membrane was estimated by electron microscopy and marker enzymes. ATPase activity was assayed in the presence of 1 mM ouabain to obtain the Mg^{2+}-ATPase (EC 3.6.1.3), and without ouabain to give total ATPase [21]. The $(Na^+ + K^+)$-ATPase activity was estimated by the difference in these activities. Succinate dehydrogenase activity

(EC 1.3.99.1) was assayed according to Green et al. [22]. NADPH-cytochrome *c* reductase activity (EC 1.6.2.4) was measured by the method of Sottacasa et al. [23]. The membrane protein content was assayed according to the procedure of Hartree [24].

Samples prepared for electron microscopy were fixed with 3 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 [25], postfixed in 1 % OsO_4, dehydrated in ethanol and embedded in Spurr's medium [26]. The sections were cut on a Sorval MT-2B ultramicrotome and mounted on uncoated 300- or 400-mesh copper grids. Staining was done with uranyl acetate [27]. followed by lead acetate [28]. The sections were examined in Hitachi HU-125E electron microscope.

Lipid analysis

Lipids were extracted from the plasma membranes with a 2 : 1 (v/v) mixture of chloroform and methanol [30]. Aliquots of the chloroform phase were taken for measurement of free and esterified cholesterol [30] and phospholipids [31]. Additional aliquots of the chloroform phase were saponified and methylated [32], and the fatty acid composition was determined using a Hewlett-Packard gas chromatograph with a 6 foot $\times$0.25 inch glass column containing 10 % Silar 10 C on Gas Chrom Q (100–200 mesh). Fatty acid identifications were accomplished by using standards obtained from Applied Sciences Laboratories. The lipid classes were separated from additional aliquots of the chloroform phase by thin-layer chromatography [33], using a solvent system of chloroform/methanol/acetic acid/water (100 : 50 : 14 : 6). The lipids were eluted from the segments of silica gel with chloroform and methanol. After saponification and methylation, the fatty acid composition of each lipid class was determined by gas-liquid chromatography as described above.

RESULTS

Plasma membrane purity

Electron micrographs indicated that the plasma membrane fraction consisted of vesicles of different sizes, and no ribosomes were seen in these preparations. A comparison of the enzymatic activities contained in the plasma membrane and crude microsomal fractions is shown in Table II. The plasma membranes are enriched in both $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activities. This is explained by the fact

TABLE II

SPECIFIC ACTIVITIES OF MARKERS ENZYMES

Values are the averages of 2 assays.

Subcellular fraction	Activity (μmol/mg protein per min)			
	Mg^{2+}-ATPase	$(Na^+ + K^+)$-ATPase	NADPH-Cytochrome *c* reductase	Succinate dehydrogenase
Plasma membrane	$180 \cdot 10^{-3}$	$33.5 \cdot 10^{-3}$	4.4	$72.0 \cdot 10^{-5}$
Microsomal fraction	$41 \cdot 10^{-3}$	$10.8 \cdot 10^{-3}$	184.8	$5.8 \cdot 10^{-2}$

that Ehrlich cells contain an Mg^{2+}-ATPase, in addition to the usual $(Na^+ + K^+)$-ATPase, on their surfaces [34]. The NADPH-cytochrome *c* reductase activity was greatly reduced in the plasma membranes, and there was almost no succinate dehydrogenase activity in this fraction.

Lipid content

A comparison of the cholesterol, cholesteryl ester and phospholipid contents of plasma membranes isolated from cells grown in mice fed the sunflower or coconut oil diets is given in Table III. The type of dietary fat and no significant effect on the amount of any of these lipids in the membrane fraction, and the cholesterol to phospholipid ratio did not change. In both cases, more than 95 % of the total cholesterol was unesterified.

TABLE III

LIPID COMPOSITION OF THE PLASMA MEMBRANE OF EHRLICH ASCITES CELLS GROWN IN MICE FED EITHER SUNFLOWER OR COCONUT OIL DIET

Values (μg/mg protein) are means ± S.E. of 3 samples.

Dietary fat	Cholesterol		Phospholipid	Phospholipid/ Free cholesterol
	Free	Esterified		
Sunflower	106.3 ± 2.3	4.1 ± 1.4	1074 ± 20	10.1 ± 0.4
Coconut	101.0 ± 8.1	5.6 ± 1.1	1015 ± 30	10.2 ± 1.1

TABLE IV

FATTY ACID COMPOSITION OF PLASMA MEMBRANE OF EHRLICH ASCITES CELLS GROWN IN MICE FED EITHER SUNFLOWER OR COCONUT OIL DIET

Fatty acid*	Composition (%)**	
	Sunflower	Coconut
14 : 0	0.6 ± 0.1	1.6 ± 0.1
16 : 0	18.9 ± 1.4	18.2 ± 0.2
16 : 1	trace***	3.1 ± 0.3
18 : 0	20.0 ± 1.3	14.4 ± 0.9
18 : 1	17.2 ± 1.0	33.1 ± 0.6
18 : 2	22.9 ± 1.1	7.7 ± 0.4
18 : 3	3.6 ± 0.1	1.4 ± 0.2
20 : 2	trace	2.0 ± 0.1
20 : 3	trace	2.2 ± 0.1
20 : 4	7.6 ± 0.9	6.6 ± 0.2
20 : 5	1.0 ± 0.1	2.8 ± 0.1
22 : 4	2.8 ± 0.7	1.2 ± 0.1
22 : 5	0.8 ± 0.4	0.8 ± 0.2
22 : 6	1.2 ± 0.2	0.6 ± 0.1

* Chain length: number of double bonds.
** Values are means ± S.E. of 3 samples.
*** < 0.5 % of fatty acids.

TABLE V

FATTY ACID COMPOSITION OF PLASMA MEMBRANE PHOSPHOLIPIDS OF EHRLICH ASCITES CELLS GROWN IN MICE FED EITHER SUNFLOWER OR COCONUT OIL DIET

Fatty acid*	Composition (%)							
	Phosphatidylethanolamine		Phosphatidylserine + phosphatidylinositol		Phosphatidylcholine		Sphingomyelin	
	Sunflower	Coconut	Sunflower	Coconut	Sunflower	Coconut	Sunflower	Coconut
14 : 0	1.0±0.2**	0.8±0.1	2.1±1.0	1.9±0.3	2.5±0.7	2.2±0.2	5.1±1.7	3.7±1.4
16 : 0	9.4±2.3	10.9±0.5	11.0±2.9	8.7±0.9	34.8±3.9	24.6±1.0	50.4±3.3	36.5±2.7
16 : 1	1.1±0.4	4.4±0.9	3.7±2.0	3.2±0.7	0.7±0.1	4.6±0.4	7.1±0.3	5.0±2.1
18 : 0	18.5±3.0	14.1±1.6	42.7±6.8	36.3±3.4	24.0±2.0	15.7±1.3	7.7±2.4	8.2±1.1
18 : 1	12.2±4.4	37.0±2.4	12.1±1.3	22.8±2.4	15.1±1.3	35.3±1.5	5.8±2.4	6.8±1.9
18 : 2	24.8±3.2	7.9±0.3	10.3±3.8	4.6±0.6	15.0±4.7	7.2±1.0	trace***	trace
18 : 3/20 : 1	trace	1.2±0.3	trace	1.0±0.2	trace	1.3±0.2	2.9±0.6	trace
20 : 4	12.2±2.2	10.4±1.0	9.4±3.9	8.9±2.5	2.7±0.9			
20 : 5		2.8±0.1	2.0±0.1	1.2±0.4	trace	5.0±1.1		
22 : 0						trace	4.1±1.2	3.9±1.0
22 : 1	trace	1.5±0.1	2.1±0.6	3.6±0.1	0.5±0.2	trace		
22 : 4	7.9±1.2	2.4±0.1	2.3±1.0	trace		trace		
22 : 5	3.3±0.2	2.4±0.5	trace	3.6±2.3		trace		
22 : 6	6.8±2.4	2.5±0.9	3.0±0.9	2.0±0.6	1.0±0.3	trace		
24 : 0							5.4±0.6	3.9±0.7
24 : 1							7.7±2.2	17.2±2.3

* Chain length: number of double bonds.

** Values are means ±S.E. of 3 samples.

*** < 0.5 % of fatty acids.

Fatty acid composition

Table IV shows the fatty acid compositions of the total lipids extracted from the plasma membranes prepared from cells grown in mice fed either sunflower or coconut oil diet. With sunflower oil, monoenoic acids accounted for only 17 % of the fatty acids, whereas they accounted for 36 % with coconut oil. Conversely, polyenoic acids of the linoleate family made up 38 % of the fatty acids with sunflower oil but only 18 % with coconut oil. Most of the decrease in polyenoic acids with coconut oil was accounted for by linoleic acid, and the arachidonic acid content was only slightly below that in the cells grown in animals fed sunflower oil. Unexpectedly, there was a slightly higher percentage of saturated fatty acids when the highly unsaturated sunflower oil was fed, 40 % as compared with 34 %.

The fatty acyl group compositions of the main phospholipid fractions isolated from the plasma membranes are given in Table V. Except in the case of sphingomyelin, there was more linoleic and less oleic acid when sunflower oil was fed. The total polyenoic fatty acid content of these fractions was higher with sunflower oil, with most of the increase being accounted for by the linoleic acid. Surprisingly, the total saturated fatty acid content of each of these phospholipid fractions also was greater with sunflower oil. Stearate was higher in all of the fractions, and palmitate was higher in every fraction except phosphatidylethanolamine.

The fatty acid composition of the small quantity of neutral lipids present in these plasma membranes is shown in Table VI. In general, the pattern was the same as that noted for the phospholipids. Neutral lipids from cells grown in mice fed sunflower oil contained much larger quantities of linoleic acid and much less oleic acid. Unlike the phospholipids, however, neutral lipids contained slightly less total saturated fatty acids than those from the cells grown in coconut oil, 46 % as compared with 50 %. Yet, their stearic acid content was somewhat greater than that of the membrane neutral lipids from the cells grown on coconut oil.

TABLE VI

FATTY ACID COMPOSITION OF PLASMA MEMBRANE NEUTRAL LIPIDS OF EHRLICH ASCITES CELLS GROWN IN MICE FED EITHER SUNFLOWER OR COCONUT OIL DIET

Fatty acid*	Composition (%)**	
	Sunflower	Coconut
14 : 0	3.1 ± 0.1	5.6 ± 0.4
16 : 0	23.7 ± 0.3	27.8 ± 3.0
16 : 1	3.0 ± 0.4	4.8 ± 0.8
18 : 0	19.3 ± 0.9	16.2 ± 1.0
18 : 1	14.9 ± 0.4	33.4 ± 1.6
18 : 2	22.7 ± 1.8	4.4 ± 1.7
18 : 3	trace***	0.9 ± 0.1
20 : 4	5.2 ± 0.6	3.1 ± 1.1
22 : 4	2.3 ± 0.3	0.5 ± 0.1
22 : 5	0.7 ± 0.0	0.5 ± 0.1
22 : 6	2.3 ± 0.2	1.1 ± 0.1

* Chain length: number of double bonds.
** Values are means ± S.E. of 3 samples.
*** < 0.5 % of fatty acids.

DISCUSSION

These findings demonstrate that the fatty acyl group composition of plasma membranes of an ascites tumor cell can be altered appreciably by changing the dietary lipid fed to the tumor-bearing host. Similar changes have been produced by modification of the dietary lipid composition in human erythrocyte [11], rat erythrocyte [12] and rat liver [13,14] plasma membranes. Therefore, with regard to variability of membrane fatty acids, the tumor cell responds in a manner similar to a nonmalignant cell. The modifications in fatty acyl group composition were not accompanied by any changes in the plasma membrane cholesterol or phospholipid content. This finding also is in agreement with observation made in nonmalignant cells [12, 35]. Only in very severe essential fatty acid deficiency [13] or following prolonged growth in a very low cholesterol culture medium [36, 37] have changes in plasma membrane cholesterol content been noted. Brenneman et al. [19] have reported an increase in the cholesterol content of the Ehrlich cell when the host is fed the coconut oil diet. The increase, however, was confined to the cell cholesteryl ester fraction. Therefore, the failure to note any change in plasma membrane cholesterol content is consistent with our previous observation [19], for the membrane cholesterol is almost entirely unesterified.

As might be expected [11, 12], the sunflower oil diet produced an increase in the polyenoic acid content of the Ehrlich cell plasma membranes. By contrast, the increase in saturated fatty acid content of all of the phospholipid classes when sunflower oil was fed was surprising. Haeffner and Privett [38] have also noted that feeding a diet high in linoleic or arachidonic acid increases the saturated fatty acid content of rat liver mitochondria. This somewhat paradoxical effect of feeding polyunsaturated fat has not been reported by other investigators and deserves further exploration. Many studies have demonstrated the importance of maintaining a specific degree of fluidity in order to maintain optimum membrane function [39–41]. Cholesterol is important in regulating membrane fluidity [36, 37, 39, 42]. The Ehrlich cell, however, apparently does not compensate for changes in membrane fatty acyl saturation by varying its cholesterol content. Instead, these cells appear to compensate for the increase in membrane polyenoic acids by raising the saturated fatty acid content of their phospholipids. This may be an important regulatory mechanism that maintains the membrane bilayer fluidity within an acceptable range for cellular function.

Being able to vary the plasma membrane fatty acyl group composition provides a powerful tool to probe the role of lipids in membrane function. In addition to our dietary approach, two other methods have been employed to modify the fatty acid composition of mammalian cell membranes. One is to incubate cells in culture with single fatty acids bound to albumin [10] or a Tween ester [7]. The other is to incubate cultured cells with phospholipid vesicles [43, 44]. In the liposome systems [43, 44], the phosphatidylcholine molecules appear to be taken up intact, perhaps by fusion with the plasma membrane lipid bilayer. The advantage of the Ehrlich cell system, as compared with the liposome methods, is that only the fatty acid composition is altered in the former. This should permit easier interpretation of any functional changes that may accompany the lipid alterations. The Ehrlich cell system also offers an important advantage over the fatty acid systems employing cultured cells [7, 43].

Because Ehrlich cells can be grown in very large quantities with a minimum of effort and expense, this system can routinely provide sufficient quantities of plasma membranes needed for sophisticated studies of membrane function.

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