Biochimica et Biophysica Acta, 450 (1976) 239—251

BBA 56880

MODIFICATION OF THE EHRLICH ASCITES TUMOR CELL NUCLEAR LIPIDS

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(Received May 4th, 1976)

Summary

The fatty acid composition of Ehrlich ascites tumor cell nuclei was different when the tumor-bearing mice were fed diets rich in either coconut or sunflower oil. When coconut oil was fed, the monoenoic fatty acid content of many of the nuclear lipids was increased and their polyenoic fatty acid content was reduced as compared with the sunflower oil diet. By contrast, only small changes were produced in the saturated fatty acid contents of the nuclear lipids. The nuclear membrane choline phospholipid, ethanolamine phospholipid and combined serine phospholipid plus inositol phospholipid fractions exhibited statistically significant changes in fatty acid composition, but the sphingomyelins were not altered appreciably by dietary lipid modification. The fatty acid composition of the small quantity of phospholipids associated with the chromatin was much more resistant to diet-induced modification. Except for sphingomyelin, the fatty acid composition of the chromatin phospholipids was different from that of the corresponding nuclear membrane phospholipids, containing much larger amounts of fatty acids having less than 16 carbon atoms. The fatty acid compositions of the nuclear triacylglycerols and cholesterol esters, which were associated almost entirely with the chromatin, were modified by the dietary lipid modifications. There were no changes in the DNA, RNA or lipid content of these nuclei. Therefore, this experimental system can be used to prepare mammalian nuclei that differ appreciably only in their fatty acyl composition.

Introduction

The nuclei of many different mammalian cells, including tumor cells [1], contain appreciable quantities of lipids [2—5]. Most of these lipids are contained in the nuclear membranes [4] and probably are involved in regulating

transport between the nucleus and the cytoplasm. Some lipids also are present in the chromatin [6], and they have been shown to bind to DNA and modify the stability of the helix [7,8].

We recently have observed that the fatty acid composition of the Ehrlich ascites tumor can be altered by feeding the tumor-bearing mice diets containing different kinds of fats [9]. This produces changes in the fatty acid composition of the plasma membrane phospholipids [10,11] and of the cholesterol esters and triacylglycerols present in the cytoplasm [12]. A major advantage of this experimental system is that very large amounts of isolated mammalian cells can be grown at relatively little expense, making the preparation of sufficient material for extensive biochemical studies feasible on a routine basis. Therefore, we thought that this system might be useful for studies of the role of lipid structure on nuclear function, provided that changes similar to those observed in the plasma membrane and cytoplasmic lipids could be produced in the nuclear lipids. The present study was done to determine the extent to which the Ehrlich cell nuclear lipids could be altered by dietary lipid modification.

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, Wisc) supplemented with either 16% sunflower oil or 16% coconut oil. Linoleic acid made up more than 70% of the fatty acids in the sunflower oil, whereas the coconut oil contained 93% saturated fatty acids [10]. Mice were fed these experimental diets for 4 weeks prior to transplantation of the tumor. The experimental diet was continued during tumor growth, and the Ehrlich cells were harvested 14 days after transplantation.

Preparation of nuclei. The procedure for transplanting and harvesting the tumor and for washing the cells has been described [13]. Nuclei were isolated from the washed Ehrlich cells by the citric acid method [14]. Light microscopy after acetocaramine staining was used to determine the number of strokes with the Dounce homogenizer required to rupture the cells [15]. The crude nuclear pellet was purified further by suspending it in 0.25 M sucrose containing 1.5% citric acid, layering this over 2 M sucrose containing 1.5% citric acid and centrifuging in an SW27 Beckman rotor for 30 min at 58 000 $\times$ *g*. After washing twice, the final pellet was suspended in Krebs-Ringer phosphate buffer, pH 7.4.

Preparation of chromatin. In order to remove the nuclear membranes, the isolated nuclei were washed twice with 1% Triton X-100 in 50 mM Tris · HCl buffer, pH 7.5, containing 25 mM KCl and 5 mM $MgCl_2$ [16]. Chromatin was prepared from the resulting pellet by the method of Swanek et al. [17]. After washing twice, the chromatin was suspended in 10 mM Tris · HCl, pH 8.0. Samples of the chromatin and isolated nuclei were fixed, embedded, stained and examined with a Hitachi HV-125E electron microscope [10].

Chemical analyses. Aliquots of the intact cell, nuclear and chromatin preparations were assayed for protein [18], RNA [19] and DNA [20]. Lipids were extracted from additional aliquots with a chloroform/methanol solution [21].

The lipids contained in the isolated chloroform phase were separated by thin-layer chromatography in a solvent system containing hexane/diethyl ether/methanol/acetic acid (90 : 20 : 2 : 3, v/v) [22]. The phospholipid band was removed, eluted and rechromatographed in a solvent system containing chloroform/methanol/acetic acid/water (25 : 15 : 4 : 2, v/v) [23]. After saponification [13] and methylation [24] of the lipids eluted from these chromatograms, their fatty acid composition was determined by gas-liquid chromatography [10]. Standards for both thin-layer and gas-liquid chromatography were obtained from Applied Science Laboratories. Aliquots of the lipid extracts also were assayed for cholesterol [25], phospholipids [26] and triacylglycerol [27].

Results

Nuclear purity

As shown in Fig. 1A, the nuclei prepared from the Ehrlich cells by these methods are essentially free of cytoplasmic contamination as judged by electron microscopy. In addition (Fig. 1B), the nuclear membrane is intact, and in some regions a double membrane structure is visible.

Table I lists the RNA and DNA contents of intact Ehrlich cells and isolated nuclei for tumors grown in mice fed either the sunflower or coconut oil diets. Although the RNA content was about the same in the cells and nuclei, the DNA content was about four times higher in the nuclei. The RNA : DNA ratio was about 3.5 times higher in the intact cells than in the nuclei. Ratios similar to these have been reported for other tumor cells [14]. The values were approximately the same in the cells and nuclei obtained from tumors grown in mice fed either the sunflower or the coconut oil diet.

Lipid content

Table II shows the lipid contents of the intact cells, isolated nuclei and chromatin. The nuclei contained 12—14% of the total lipids present in the cells. The chromatin contained about 3% of the lipids present in the intact cells and 23—25% of the lipids contained in the intact nucleus. Considerable variations were noted, however, in the distributions of the individual lipids. For example, the nuclei contained only about 5% of the triacylglycerols present in the cells, and all of the nuclear triacylglycerols were recovered in the chromatin fraction. All of the nuclear cholesterol esters also were present in the chromatin, but only 10% of the nuclear free cholesterol was associated with the chromatin fraction. The ratio of free cholesterol to phospholipids was essentially the same in the nuclei and intact cells, but it was four times higher in the chromatin fraction. Significant differences were observed in the triacylglycerol contents of the intact cells but not in either the nuclear of chromatin fractions. Likewise, there were no differences in the amounts of cholesterol or phospholipids in either the nuclear or chromatin fractions from the cells grown on sunflower as compared with coconut oil.

Fatty acid composition

Table III shows the fatty acid composition of the choline-containing phospholipids in the nuclear and chromatin fractions of the Ehrlich cell. Since only

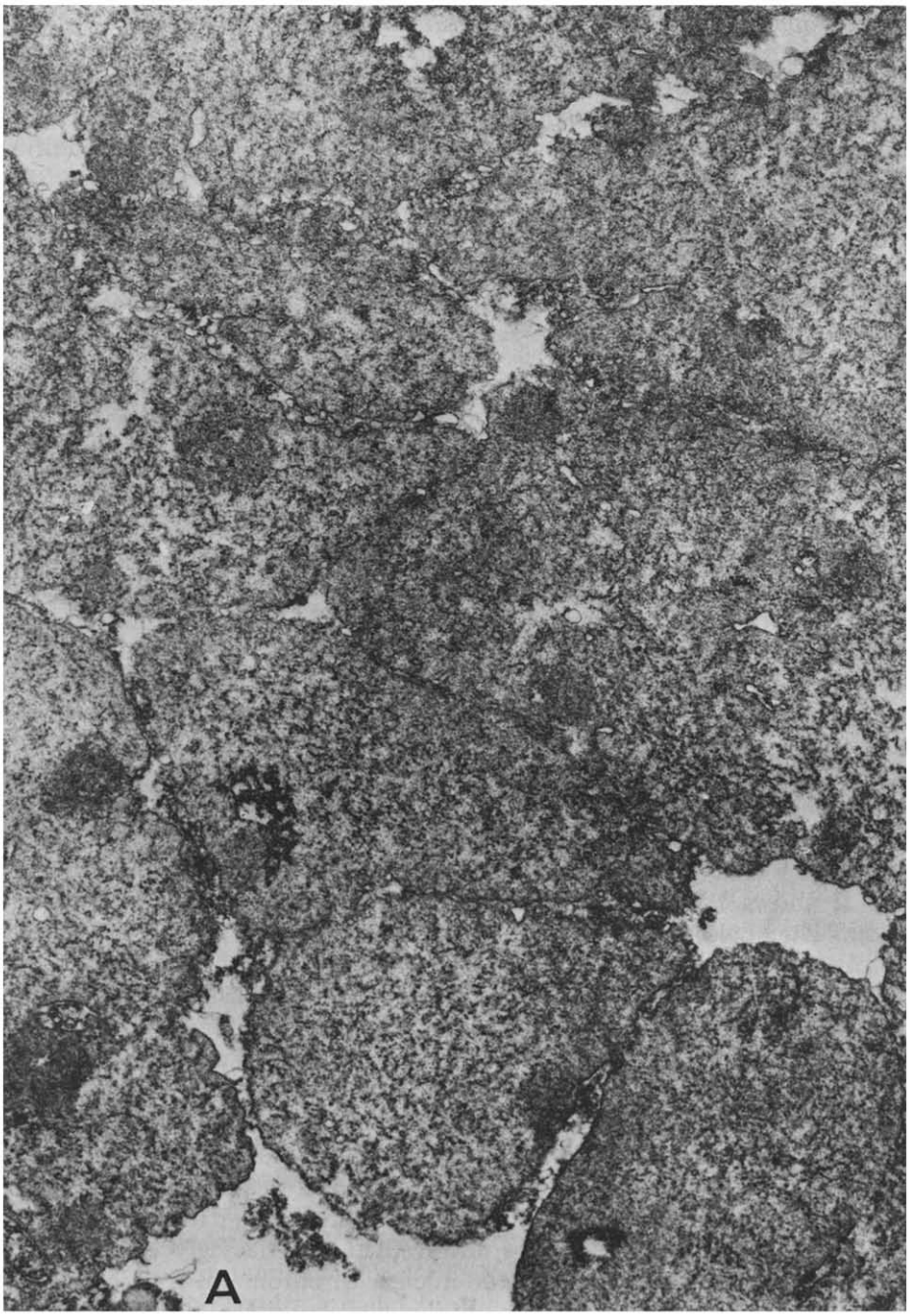

Fig. 1. See legend on opposite page.

3% of the total nuclear phospholipids were recovered in the chromatin fraction (Table II), the data for nuclear phospholipids are for the most part indicative of the nuclear membrane phospholipids. There was little difference in the saturated fatty acid contents of the nuclear choline phospholipids obtained

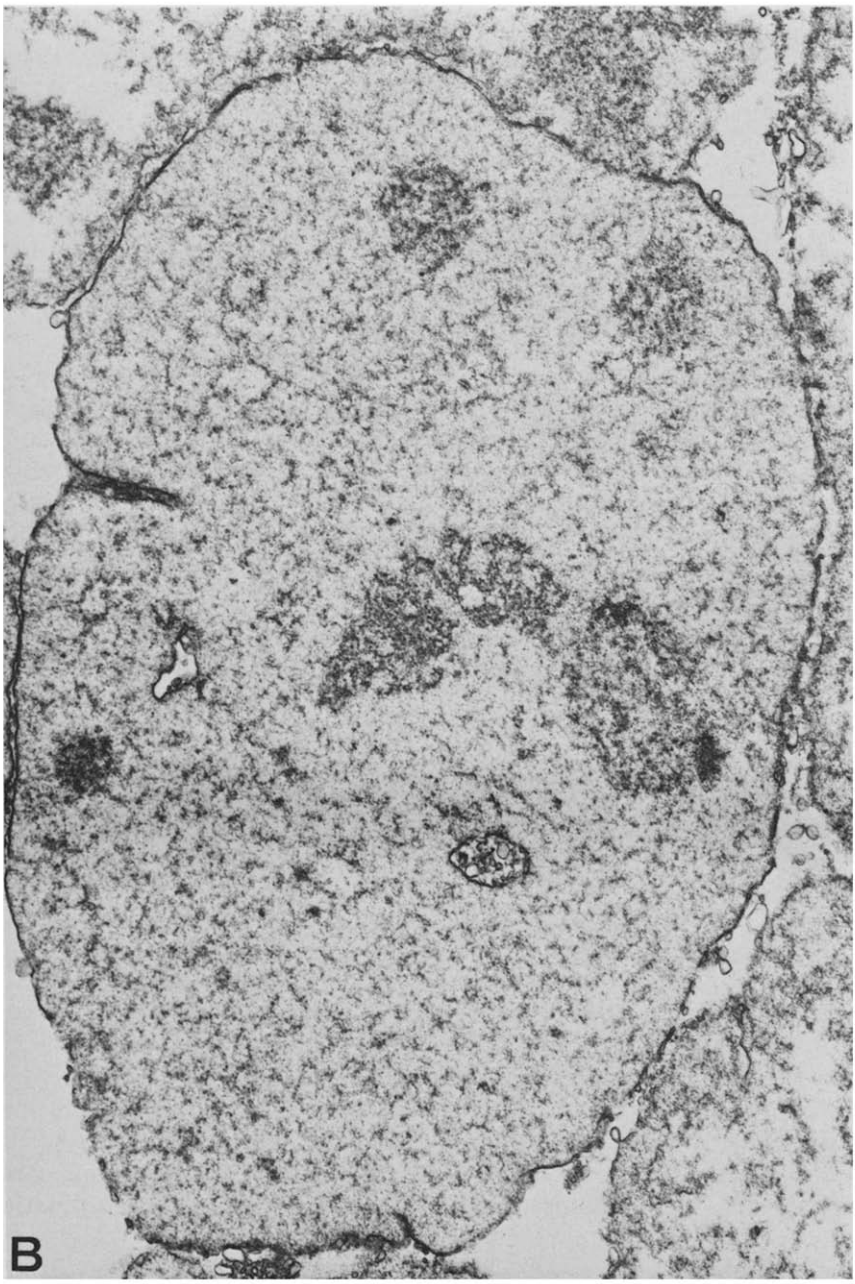

Fig. 1. (A) Electron micrograph of a nuclear pellet isolated from Ehrlich ascites tumor cells, X 10 500. No other contaminating structures are observed. (B) Electron micrograph of a single nucleus, X 15 000. Both the outer and inner nuclear membranes remain intact throughout much of the nucleus.

from cells grown on sunflower as compared with coconut oil. A number of differences were noted, however, in the unsaturated fatty acid composition. With sunflower oil, the nuclei contained 19% monoenoic and 45% polyenoic fatty acid. With coconut oil, they contained 35% monoenoic and only 29% polyeno-

TABLE I

COMPARISON OF RNA AND DNA CONTENTS OF NUCLEI AND INTACT CELLS GROWN IN MICE FED EITHER THE SUNFLOWER OR COCONUT OIL DIETS

Values are means ± S.E. of three samples.

Nucleic acid	Cells		Nuclei	
	Sunflower	Coconut	Sunflower	Coconut
RNA (mg/mg protein)	0.08 ± 0.01	0.07 ± 0.05	0.08 ± 0.01	0.09 ± 0.01
DNA (mg/mg protein)	0.07 ± 0.01	0.06 ± 0.01	0.26 ± 0.03	0.24 ± 0.03
RNA : DNA ratio	1.15 ± 0.07	1.09 ± 0.05	0.33 ± 0.04	0.34 ± 0.03

ic fatty acids. These changes were due primarily to differences in the 18 : 1 and 18 : 2 contents. In spite of the large differences in polyenoic fatty acid content, the amount of arachidonic acid in the choline phospholipid from both nuclei was similar.

As compared with the choline phospholipids of the intact nucleus, the small amounts isolated with the chromatin fraction were much more resistant to dietary lipid modification. There were no differences in either the 18 : 1 or 18 : 2 contents of the chromatin choline phospholipids isolated from the cells grown on either sunflower or coconut oil. In both samples, monoenoic fatty acids made up 34% of the total. There were slightly smaller amounts of polyenoic and somewhat larger amounts of saturated fatty acids in the chromatin choline phospholipids from the cells grown on coconut oil, but this change was accounted for primarily by an increase in saturated fatty acids containing 8—14 carbon atoms.

With both diets, the chromatin choline phospholipids contained lots of fatty acids having less than 16 carbon atoms. These medium-chain fatty acids comprised less than 3% of the fatty acids in the choline phospholipids of the intact nucleus but from 24 to 33% of those in the chromatin choline phospholipids. In addition, the chromatin choline phospholipids contained 7—19% more saturated and 11—23% less polenoic fatty acids than the intact nuclear choline phospholipids.

There were essentially no dietary lipid-induced changes in fatty acid composition of the sphingomelins isolated from either the intact nuclei or the chromatin. Moreover, the chromatin sphingomelins had fatty acid compositions quite similar to those of the intact nucleus. A 7% increase in fatty acids containing less than 16 carbon atoms, however, was observed in the chromatin fraction.

As shown in Table IV, the same overall pattern of results noted with the choline phospholipids also was observed for the other phospholipid fractions that were isolated, the ethanolamine phospholipid and the combined serine phospholipid plus inositol phospholipid fractions. The fractions isolated from the intact nuclei contained less monoenoic and more polyenoic fatty acid when the cells were grown in mice fed the sunflower oil diet. These differences were accounted for primarily by changes in the 18 : 1 and 18 : 2 contents. Only small differences in the saturated fatty acid contents were produced by the two diets. As noted for the choline phospholipids, the ethanolamine

TABLE II

COMPARISON OF THE LIPID CONTENTS OF INTACT CELLS, NUCLEI AND CHROMATIN FROM TUMORS GROWN IN MICE FED EITHER THE SUNFLOWER OR COCONUT OIL DIETS

Values (μg/mg DNA) are the mean ± S.E. of three samples.

Sample	Dietary fat	Cholesterol		Phospholipids *	Triacylglycerols	Free cholesterol/ phospholipid
		Free	Esterified			
Whole cell	Sunflower	124 ± 5.3	108 ± 6.2	912 ± 7.4	1166 ± 42 **	0.14 ± 0.02
	Coconut	121 ± 5.9	121 ± 6.2	961 ± 29	856 ± 44 **	0.13 ± 0.01
Nucleus	Sunflower	26.0 ± 3.8	5.6 ± 0.1	183 ± 11	57.8 ± 3.0	0.14 ± 0.03
	Coconut	23.1 ± 5.0	7.8 ± 0.6	198 ± 13	48.5 ± 5.8	0.12 ± 0.02
Chromatin	Sunflower	2.2 ± 0.2	4.7 ± 0.6	4.7 ± 0.6	55.1 ± 2.2	0.48 ± 0.01
	Coconut	2.7 ± 0.1	7.6 ± 0.4	4.7 ± 0.6	48.4 ± 3.0	0.59 ± 0.08

* This fraction is made up of 42% choline phospholipids, 29% ethanolamine phospholipids, 16% sphingomyelins and 13% of serine phospholipids plus inositol phospholipids. The values for the intact cell and nuclear phospholipid fractions were the same. There was too little phospholipid in the chromatin fraction to make these measurements.

** Values are significantly different ($P < 0.05$).

TABLE III

FATTY ACID COMPOSITION OF NUCLEAR AND CHROMATIN CHOLINE PHOSPHOLIPIDS AND SPHINGOMYELINS OF CELLS GROWN IN MICE FED EITHER THE SUNFLOWER OR COCONUT OIL DIET

Fatty acid *	Composition (%)							
	Choline phospholipids				Sphingomyelin			
	Nucleus **		Chromatin ***		Nucleus **		Chromatin ***	
	Sunflower	Coconut	Sunflower	Coconut	Sunflower	Coconut	Sunflower	Coconut
<14 : 0 †	trace	0.6 ± 0.1	10.0	15.0	3.2 ± 1.0	2.5 ± 0.6	5.5	6.4
14 : 0	1.1 ± 0.1	1.7 ± 0.1	6.3	8.4	7.0 ± 0.6	7.5 ± 0.8	8.4	8.0
14 : 1	0.6 ± 0.1	0.6 ± 0.1	7.6	9.7	2.8 ± 0.5	2.8 ± 0.3	5.9	5.5
16 : 0	17.6 ± 0.3	17.2 ± 1.3	12.8	15.3	28.7 ± 0.9	30.4 ± 1.6	12.6	10.8
16 : 1	2.4 ± 0.1	4.3 ± 0.2	11.0	8.8	7.7 ± 0.8	8.2 ± 0.9	8.4	7.6
18 : 0	14.1 ± 0.4	10.1 ± 0.3	7.7	7.8	7.6 ± 0.8	8.7 ± 1.5	7.4	7.0
18 : 1	15.3 ± 0.8	28.4 ± 1.9	14.2	14.8	14.0 ± 0.8	17.7 ± 2.2	14.1	12.6
18 : 2 ††	17.6 ± 0.8	10.9 ± 0.3	9.2	9.0	5.3 ± 0.2	5.1 ± 0.4	8.7	9.6
18 : 3	1.6 ± 0.1	2.4 ± 0.2	2.0					
20 : 2	3.0 ± 0.2	1.3 ± 0.2	3.8	3.6				
20 : 4	7.5 ± 0.3	8.0 ± 0.5	7.2	2.6				
22 : 0		0.8 ± 0.1	3.2	2.4				
22 : 1	0.9 ± 0.1	1.5 ± 0.2	0.7	0.8	0.7 ± 0.1		4.8	4.7
22 : 4	2.8 ± 0.2	2.1 ± 0.3						
22 : 5	trace	1.1 ± 0.3	2.0	2.5				
22 : 6	3.0 ± 0.2	2.7 ± 0.6						
24 : 0					2.5 ± 0.1	1.5 ± 1.0	8.2	10.2
24 : 1					2.9 ± 1.2	3.6 ± 1.4	2.8	2.5
Others	2.5	6.3	2.3		17.7	11.9	13.3	15.5

* Chain length: number of double bonds.

** Values are the mean ± S.E. of 4 samples.

*** Values are the average of 3 samples.

† In the intact nucleus, this fraction is composed almost entirely of 12 : 0. In the chromatin, it contains 11% 8 : 0, 42% 10 : 0 and 47% 12 : 0.

†† This chromatography column did not separate 18 : 2 from 20 : 0. Therefore, it is possible that the 18 : 2 fraction contained 20 : 0, particularly in the sphingomyelins. It has been reported that the 18 : 2 fraction of liver plasma membrane sphingomyelin contains 25—55% 20 : 0 [30].

TABLE IV

FATTY ACID COMPOSITION OF NUCLEAR AND CHROMATIN ETHANOLAMINE PHOSPHOLIPIDS AND COMBINED SERINE PHOSPHOLIPID PLUS INOSITOL PHOSPHOLIPID FRACTIONS OF CELLS GROWN IN MICE FED EITHER THE SUNFLOWER OR COCONUT OIL DIET

Fatty acid *	Composition (%)							
	Ethanolamine phospholipids				Serine phospholipids + inositol phospholipids			
	Nucleus **		Chromatin ***		Nucleus **		Chromatin ***	
	Sunflower	Coconut	Sunflower	Coconut	Sunflower	Coconut	Sunflower	Coconut
<14 : 0 †	trace	0.7 ± 0.2	11.6	14.4	0.7 ± 0.1	0.8 ± 0.1	11.4	17.7
14 : 0	0.6 ± 0.1	1.2 ± 0.4	9.4	10.4	0.9 ± 0.1	2.0 ± 0.4	7.8	10.4
14 : 1	trace	0.6 ± 0.2	8.2	3.9	trace	0.6 ± 0.1	6.4	9.4
16 : 0	9.4 ± 0.5	9.5 ± 1.3	19.0	23.1	3.9 ± 0.4	8.1 ± 1.6	13.9	19.1
16 : 1	1.5 ± 0.1	2.8 ± 0.3	8.8	8.8	1.2 ± 0.2	2.1 ± 0.4	6.5	12.2
18 : 0	16.7 ± 0.8	12.0 ± 1.2	9.0	10.8	37.5 ± 1.7	35.3 ± 2.7	8.9	8.0
18 : 1	15.3 ± 0.7	26.4 ± 2.4	14.9	18.8	8.0 ± 0.3	15.1 ± 1.5	13.6	14.9
18 : 2	24.5 ± 1.3	10.0 ± 0.6	4.5	3.7	10.7 ± 0.4	4.7 ± 0.5	7.6	4.1
18 : 3	1.2 ± 0.2	2.0 ± 0.5	0.8		0.6 ± 0.2		1.5	
20 : 2	2.5 ± 0.2	1.1 ± 0.4			1.3 ± 0.3		1.3	
20 : 4	11.4 ± 0.4	12.3 ± 0.8	2.5	1.4	23.7 ± 1.3	19.9 ± 1.6	9.4	trace
22 : 1	1.0 ± 0.1	1.6 ± 0.4	0.9		2.5 ± 0.1	2.8 ± 0.2	3.2	0.6
22 : 4	5.8 ± 0.1	3.8 ± 0.5			2.5 ± 0.2	1.2 ± 0.1		trace
22 : 5	1.2 ± 0.3	1.6 ± 0.6	1.2	1.0	0.6 ± 0.3	trace	2.8	1.0
22 : 6	6.2 ± 0.8	5.7 ± 1.3			2.2 ± 0.4	0.8 ± 0.1		
Others	2.7	8.7	9.2	3.7	3.5	6.6	5.7	2.6

* Chain length: number of double bonds.

** Values are the mean ± S.E. of 4 samples.

*** Values are the average of 3 samples.

† In the intact nucleus, this fraction was composed almost entirely of 12:0. In the chromatin, it contained 13% 8:0, 53% 10:0 and 34% 12:0.

TABLE V

FATTY ACID COMPOSITION OF NUCLEAR TRIACYLGLYCEROLS AND CHOLESTEROL ESTERS OF CELLS GROWN IN MICE FED EITHER THE SUNFLOWER OR COCONUT OIL DIET

Fatty acid *	Composition (%) **			
	Triglycerides		Cholesterol esters	
	Sunflower	Coconut	Sunflower	Coconut
12 : 0	trace	3.1 ± 0.6	3.6 ± 0.2	3.3 ± 0.8
14 : 0	1.2 ± 0.1	4.7 ± 0.5	10.5 ± 0.8	12.3 ± 0.9
14 : 1	trace	1.0 ± 0.1	4.6 ± 0.6	3.9 ± 0.6
16 : 0	13.4 ± 0.3	18.3 ± 0.9	23.6 ± 1.2	25.0 ± 2.5
16 : 1	2.1 ± 0.1	6.2 ± 0.4	6.7 ± 0.3	8.5 ± 0.5
18 : 0	9.9 ± 0.3	7.6 ± 0.9	7.2 ± 1.1	5.8 ± 0.4
18 : 1	19.1 ± 0.3	36.4 ± 1.0	12.1 ± 0.6	17.3 ± 1.1
18 : 2	33.5 ± 0.6	9.7 ± 0.4	12.1 ± 1.3	4.8 ± 0.5
18 : 3	2.1 ± 0.2	2.8 ± 0.4	1.0 ± 0.2	1.7 ± 0.4
20 : 2	4.7 ± 0.2	1.0 ± 0.2	trace	1.5 ± 0.3
22 : 0		0.6 ± 0.1	2.9 ± 0.3	0.8 ± 0.2
22 : 1	1.1 ± 0.4		1.1 ± 0.1	1.0 ± 0.3
20 : 4	4.7 ± 0.2	2.8 ± 0.4	4.3 ± 0.6	3.1 ± 0.6
22 : 4	2.5 ± 0.3	0.8 ± 0.1	2.5 ± 0.3	1.3 ± 0.6
22 : 5	trace		0.9 ± 0.1	1.5 ± 0.6
22 : 6	2.7 ± 0.3	0.9 ± 0.1	2.2 ± 0.5	1.9 ± 0.8
Others	3.0	4.1	4.7	6.3

* Chain length: number of double bonds.

** Values are the mean ± S.E. of four samples.

phospholipids associated with the chromatin were much more resistant to diet-induced changes in fatty acid composition. Likewise, the fatty acid composition of the chromatin ethanolamine phospholipids was considerably different from that of the nuclear ethanolamine phospholipids. The fatty acid composition of the chromatin serine phospholipid plus inositol phospholipid fraction also was much different from that of the nuclear fractions. In this case, however, the chromatin fraction was more responsive to dietary lipid modification than either the chromatin choline phospholipids or ethanolamine phospholipids. As observed with the choline phospholipids, these chromatin fractions also contained a great deal more medium-chain fatty acids than the corresponding glycerophospholipid fractions of the intact nucleus.

The fatty acid compositions of the triacylglycerols and cholesterol esters from these nuclei are listed in Table V. Since essentially all of these neutral lipid esters are present in the chromatin (Table II), we did not subfractionate the nuclear material for these assays. Large diet-induced changes in fatty acid composition were noted in the triacylglycerol. The nuclear triacylglycerols isolated from mice fed the sunflower oil diet contained 22% monoenoic and 50% polyenoic fatty acids, as compared with 44 and 18%, respectively, in the cells grown on coconut oil. Somewhat less saturated fatty acid also was present in the nuclear triacylglycerols of the cells grown on sunflower oil, 25% as compared with 34% on coconut oil. Much smaller changes occurred in the cholesterol esters. Their saturated fatty acid contents were approximately the same with both diets. Although the cholesterol esters of the cells grown on sun-

flower oil contained less monoenoic and more polyenoic fatty acids, the differences as compared with the cells grown on coconut oil were 3.5—4.5 times smaller than in the triacylglycerol fraction.

Discussion

These results demonstrate that the fatty acyl composition of many of the lipids in the Ehrlich ascites cell nucleus can be altered appreciably by changing the dietary lipid fed to the tumor-bearing host. Although the general trend of the nuclear changes is similar to those observed previously in the plasma membrane [10], the magnitude of the differences is somewhat smaller in the nucleus. The main effect was on the saturation of the fatty acyl chains. The change in saturation was accounted for primarily by replacement of dienoic by monoenoic fatty acids when the diet containing the highly saturated coconut oil was fed. The changes in the saturated fatty acid contents of the nuclear lipids were small and variable. For example, the saturated fatty acid content decreased from 33 to 30% in the choline phospholipids but increased from 43 to 50% in the combined serine phospholipid plus inositol phospholipid fraction when the coconut oil diet was fed. The amounts of the various lipid classes in the nucleus were not changed appreciably by these dietary lipid modifications. Therefore, this experimental system can be employed to study the specific effects of fatty acid saturation on the function of mammalian nulcei.

As compared with the glycerophospholipids of the intact nucleus, the small amounts of these phospholipids present in the isolated chromatin were much more resistant to diet-induced changes in fatty acyl group composition. In addition, the fatty acid compositions of the chromatin phosphoglycerides were quite different from those of the corresponding fractions of the intact nucleus. One of the most striking differences was the large amount of saturated fatty acids containing 8—14 carbon atoms in the chromatin glycerophospholipids. These findings suggest that the chromatin glycerophospholipids may represent a separate pool from the bulk of the glycerophospholipids present in the nucleus.

It has been observed that ethanolamine plasmalogens of the rat testes contain highly polyunsaturated fatty acids at the 2-acyl position which are very resistant to diet-induced changes in composition [28]. We have not separated these plasmalogens from the ethanolamine phospholipid fraction in the present work. Therefore, it is possible that the diacyl ethanolamine phospholipids actually underwent greater diet-induced changes than appear to have occurred from our data, assuming that the plasmalogens which we have included in this fraction also are resistant to diet-induced changes. Similarly, it is possible that we also have underestimated the extent of the changes in the other diacyl phospholipids, for the alkyl ether species were not separated from any of the glycerophospholipid fractions.

Several of our quantitative lipid values differ to some extent from those reported for other nuclei. For example, less than 1% of the cholesterol in bovine thyroid nuclei is reported to be esterified [5], whereas we found 15—20% of the cholesterol in Ehrlich cell nuclei in the form of cholesterol esters. A free to esterified cholesterol ratio of 7.4 has been reported for calf thymus nuclei

[6], whereas we found that the Ehrlich cell values ranged from only 3 to 4.6 (Table II). The value of 1.5—3.7 reported for this ratio in the calf thymus chromatin [6] also is considerably higher than the Ehrlich cell values of 0.4—0.5 that we have observed. Although the average value for this ratio in rat liver chromatin, about 1.2, also is much higher, values comparable to ours (0.5) were found in rat liver chromatin if the rats were kept in the dark for 10—12 h before they were killed [29]. The differences between the values that we and others have observed may be due to either species variations, tissue variations, or the methods used to isolate the nuclei. Problems have been reported with the citric acid method, such as removal of the outer nuclear membrane [2]. We found by electron microscopy, however, that cytoplasmic lipid droplets remained adherent to the isolated nuclei unless citric acid was used and was followed by further centrifugations through high concentrations of sucrose. This additional purification probably accounts for the fact that we found only 12—14% of the cell lipids in the isolated nuclei whereas Bálint and Holczinger [1] observed that the nucleus contained 22% of the Ehrlich ascites cell lipids. It should be noted that the double membrane structure remains in some parts of the nucleus in our nuclear preparations (Fig. 1).

In conclusion these results suggest the possibility that some functions of a tumor cell nucleus might be modulated by the kind of dietary fat consumed by the tumor-bearing host. The functions most likely to be influenced are those involving the permeability or physical properties of the nuclear membrane. Modification of these processes could affect the growth rate or susceptability of the tumor to chemotherapy. Therefore, further study of this problem appears to be warranted.

Acknowledgements

This work was supported by research grant HL 14,781 and postdoctoral fellowship HL 07007 from the National Heart and Lung Institute. We thank Mr. Kenneth C. Moore for assistance with electron microscopy.

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