Lipids of whole cells and plasma membrane fractions from Balb/c3T3, SV3T3, and concanavalin A-selected revertant cells[1]

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Abstract The lipid composition of Balb/c3T3, SV3T3, and the concanavalin A-selected SV3T3 revertant cells has been analyzed at the whole cell and plasma membrane levels. In comparison to untransformed 3T3 whole cells, SV3T3 cells showed an unchanged content of triacylglycerols, free fatty acids, and glycerylether diesters but a lower concentration of total phospholipids, while no significant difference was found in the phospholipid composition. Whole SV3T3 revertant cells exhibited a lipid composition similar to that in untransformed 3T3 cells with the exception of a higher proportion of sphingomyelin. Analysis of isolated plasma membranes did not reveal any significant differences in the cholesterol to phospholipid molar ratio between 3T3 and SV3T3 or SV3T3 revertant cells. The major changes in the acyl chain pattern SV3T3 compared with whole 3T3 cells consisted of an increase of oleic and palmitoleic acids coupled with a decrease of C_{20} and C_{22} polyunsaturated acids in phosphatidylethanolamine and phosphatidylcholine; an increase of oleic acid was also evident in SV3T3 phosphatidylinositol plus phosphatidylserine. An increase of palmitoleic and oleic acids together with a decrease of arachidonic acid was also found in phosphatidylethanolamine of SV3T3 plasma membranes; the only change in SV3T3 plasma membrane phosphatidylcholine was an increase of oleic acid. An increase of monoenoic acids together with a decrease of arachidonic acid was also found in phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol plus phosphatidylserine of SV3T3 revertant cells at the level of both whole cells and plasma membranes. —**Ruggieri, S., R. Roblin, and P. H. Black.** Lipids of whole cells and plasma membrane fractions from Balb/c3T3, SV3T3, and concanavalin A-selected revertant cells. *J. Lipid Res.* 1979. **20:** 772–783.

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Interest in the lipids of tumor cell membranes has been stimulated by an increasing awareness of the involvement of different lipid types in a broad spectrum of cellular function. Several differences in the chemistry and in the metabolism of various lipid classes of different tumors have already been noticed in the last decade [see symposium edited by Wood (1)]. However, the relevance of these lipid differences to the altered behavior of malignant cells is still uncertain, due to the heterogeneity of cell populations, the large differences in growth rates and degree of differentiation among the tumors examined, and, in many cases, to the difficulties in the definition of a proper control tissue. These problems have been partially resolved by the development of established cell lines with controlled growth characteristics and by the availability of their transformed counterparts produced by oncogenic viruses (2, 3), chemical carcinogens (4), and X-ray irradiation (5).

In vitro cell systems have been intensively studied with regard to the molecular changes in plasma membranes associated with transformation. Of the various membrane components taken into consideration, glycosphingolipids have received special attention by several laboratories. These studies have shown that transformation by tumor viruses is accompanied by reduction and/or simplification of the more complex glycolipids (6, 7).

Until recently, little attention was devoted to phos-

Abbreviations: PBS, phosphate-buffered saline; EGTA, ethylene-bis(oxyethylenenitrilo)tetraacetic acid; FCS, fetal calf serum; GEDE, glycerylether diesters; TG, triacylglycerols; FFA, free fatty acids; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; SP, sphingomyelin; PA, phosphatidic acid; LPC, lysolecithin; SEM, standard error of the mean.

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pholipids and neutral lipids, although they might well be involved in the surface properties and metabolic derangements observed in virus-transformed cells. Recently there have been a few reports covering partial aspects of the lipid composition of viral-transformed cells at the level of whole cells (8–13) and plasma membranes (14–16).

The present investigation deals with the lipid composition of whole cells and plasma membranes of SV40-transformed Balb/c3T3 cells (SV3T3 cells) as compared to untransformed cells. The study also includes concanavalin A-selected SV3T3 revertant cells (SV3T3 Rev cells), which exhibit contact inhibition similar to untransformed cells (17). Due to its growth properties, this variant of SV3T3 cells offers the opportunity to discriminate between the molecular changes linked to transformation per se and those associated with density-dependent inhibition of growth. A detailed lipid analysis of the fetal calf serum used throughout this study is also included, since there is evidence that the lipids contained in growth media are the main source of the cellular lipid requirement (18–20) and influence the fatty acid composition of cultured cells grown therein (18–21).

MATERIALS AND METHODS

Cell culture

The cells used in these studies were Balb/c3T3 cells (clone A31), SV40-transformed Balb/c3T3 cells (clone SVT2), both obtained from Dr. Stuart Aaronson, and concanavalin A-selected Balb/c SV3T3 revertant cells (SV3T3 Rev cells) (17). The cells were grown either in 32-ounce bottles, in 425-mm-length Bellco Roller bottles, or in 100 × 15 mm Falcon dishes, at 37°C in an atmosphere of 5% CO_2 in air, using Eagle's minimal essential medium supplemented with a 4-fold concentration of vitamins and essential amino acids (MEM × 4), 10% fetal calf serum (Grand Island Biological Company, Grand Island, NY), glutamine (2 mM), 250 U/ml penicillin, and 250 μg/ml streptomycin sulfate.

The cell lines were routinely subcultivated using a 0.25% trypsin solution. The cells were seeded at about 1×10^6 cells per bottle or dish, changed to fresh medium the day after plating and then every other day, and harvested at confluence. In different experiments the final saturation densities of Balb/c3T3, SV3T3, and SV3T3 Rev cells were, respectively, $2–6 \times 10^4$, $11–30 \times 10^4$, and $4–11 \times 10^4$ cells per cm^2. The cell counts were determined using a hemocytometer or an automatic laser beam cell counter (Cytograf, Bio/Physics System, Inc., Mahopac, NY) and the cell viability was checked by vital staining after the cell suspensions were exposed for 5 min to an equal volume of a 0.4% trypan blue solution in 0.1 M phosphate-buffered saline, pH 7.2, (PBS). All the cells used in this study were at their 5th to 30th passage levels in our laboratory.

Harvesting of cells

When used for whole cell lipid analysis, the cells were harvested at confluence as follows. The growth medium was decanted and the cell layers were gently washed twice with PBS, then the cells were released by incubation, with continuous shaking, at 37°C in 10 ml of PBS containing 0.02% EGTA (ethylenebis(oxyethylenenitrilo)tetraacetic acid) (Eastman Organic Chemicals, Rochester, NY). The cell suspensions were centrifuged and washed twice by resuspension and centrifugation in PBS. The PBS suspensions of the final pellets were adjusted to $10–20 \times 10^6$ cells per ml and sonicated (Raytheon sonicator, Waltham, MA, or Labsonic 1510, Braun, Melsungen, West Germany).

In the case of lipid analyses of plasma membranes, the cells grown in 100- or 150-mm plastic dishes were washed twice with warm PBS and removed by incubation on a rotary shaker at 37°C with 6 ml/plate of a once-crystallized trypsin (Sigma Chemical Co., St. Louis, MO) solution in warm PBS (10 μg/ml). Under these conditions removal of the cells was rather rapid (5 min) and yielded suspensions with a high percentage of viable cells (not less than 90%). Trypsinization was inhibited by adding 6 ml/plate of a 2% FCS dilution in PBS followed by gentle pipetting of the cell suspensions. The cell suspensions were transferred from the dishes into plastic tubes and centrifuged for 10 min in an International PR6000 centrifuge at 1500 rpm with a 269 rotor head. The cells were washed twice by suspension in 0.14 M NaCl and centrifugation.

Plasma membrane isolation

The washed cells, resuspended in a 0.001 M $ZnCl_2$ solution at $150–300 \times 10^6$ cells/ml, were swollen for 15 min at room temperature, transferred into a large Dounce homogenizer, and cooled in an ice bath. After 5 min the cells were ruptured using a tight-fitting pestle (pestle B, Kontes, Vineland, NY). Cell rupturing was continuously controlled by a phase-contrast microscope and stopped when 70% of the cells were broken, which generally occurred after 50–60 strokes for all of the different cell types. The homogenized cell suspensions were centrifuged for 15 min at 1400 rpm in an International PR6000 centrifuge with rotor 269. The pellets were resuspended in 0.001 M $ZnCl_2$ solution and centrifuged as before. The final pellet

containing surface membranes and nuclei was resuspended with 12 ml of upper phase of the dextran–polyethyleneglycol two-phase system prepared according to the method of Brunette and Till (22). After adding an equal volume of the lower phase, the mixture was pipetted up and down several times and centrifuged for 15 min at 9500 rpm in an International B60 centrifuge with a SB110 rotor. This procedure was repeated two more times with the plasma membrane fractions collected at the interphase, each time using new upper and lower phases (12 ml each) of the Brunette and Till system (22). The plasma membrane fraction was then transferred into 50-ml plastic tubes, resuspended in four times as much 0.14 M NaCl solution and centrifuged for 15 min at 2000 rpm in an International PR6000 centrifuge with a 269 rotor; centrifugation and resuspension in the same solution were repeated twice in order to obtain a complete removal of the contaminating dextran and polyethyleneglycol. 5′-Nucleotidase activity, assayed according to Avruch and Wallach (23) on portions of pellet, showed a 40-fold increase over that of the whole cell homogenate. Portions of the final pellets were occasionally fixed and processed for examination by electron microscopy. The electron microscope examination, kindly performed by Dr. E. Raviola (Department of Anatomy, Harvard Medical School), revealed long sheets of plasma membranes with only a slight contamination by nuclei.

Extraction and analyses of lipids

Total lipids from the sonicated whole cells and plasma membrane fraction were extracted by the method of Folch, Lees, and Sloane Stanley (24). Complete extraction of the lipids from plasma membrane fractions required that the residues be re-extracted with 50 ml of chloroform–methanol 1:2. This extract, combined with the preliminary extract of plasma membranes was concentrated in a small volume and purified on a Sephadex G-25 column (25). Lipids of fetal calf serum (FCS) used throughout this study were extracted (24) and submitted to lipid analysis.

Total lipids were fractionated into neutral lipids and polar lipids by silicic column chromatography (Unisil 200–325 mesh, Clarkson Chemical Co., Williamsport, PA) using chloroform and methanol as eluents. The neutral lipids were fractionated by thin-layer chromatography on 0.25-mm precoated plates of Silica Gel H (Applied Science Laboratories, State College, PA, or Merck, Darmstadt, West Germany) using the solvent system hexane–diethyl ether–acetic acid 85:15:1. After plates were sprayed with 0.1% dichlorofluorescein in ethanol and visualized under ultraviolet light, cholesteryl esters, glycerylether diesters (GEDE), triacylglycerols (TG), free fatty acids (FFA), and free cholesterol were identified by comparison with pure standards and eluted from the absorbent with chloroform.

Phospholipids (500–800 μg) were spotted on 0.25-mm Silica Gel H precoated plates activated for 1 hr at 110°C before use, and fractionated by two-dimensional thin-layer chromatography, using chloroform–methanol–ammonia 90:45:11 in the first direction, and chloroform–methanol–acetic acid–water 90:40:12:2 in the second direction. Under these conditions, phospholipids were fractionated into diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingomyelin (SP), and lysolecithin (LPC); phosphatidylinositol (PI) and phosphatidylserine (PS), not always satisfactorily separated, were combined and analyzed together. In some instances, the fractionation of whole cell phospholipids resulted in the separation of very small fractions from DPG. These fractions, which exhibited a migration rate similar to that reported by Brotherus and Renkonen (26) for bis-phosphatidic acid and its partially deacylated derivatives, were not further characterized and were recombined with the DPG fraction.

After visualization with Rhodamine 6G or dichlorofluorescein under ultraviolet light, the phospholipid fractions were localized and scraped into vials for the determination of phosphorus content or fatty acid composition. To confirm the absence of certain phospholipid classes, the chromatograms were also sprayed with the molybdenum blue reagent (27) that would effectively reveal as low as 0.8 μg of phospholipids.

Analytical determinations

The protein content of whole cells and plasma membranes was determined by the method of Lowry et al. (28) with bovine serum albumin from Sigma as a standard. The average protein contents ±SEM in whole Balb/c3T3, SV3T3, and SV3T3 Rev cells were 289 ± 9.5, 240 ± 10.6, and 381 ± 16.6 μg per 1×10^6 cells, respectively.

Total cholesterol was measured colorimetrically (29) on total lipid extracts. Total phospholipids were determined from the lipid-phosphorus ×25 as estimated in total lipid extracts by the method of Martin and Doty (30) after digestion with sulfuric acid–perchloric acid 3:2.

In a few experiments, cholesterol and phospholipids were determined in the lipid extracts of both the homogenate of EGTA-detached cells and the $ZnCl_2$-homogenate of trypsin-detached cells. The

results of these experiments showed that the exposure to trypsin solution and $ZnCl_2$ solution does not interfere with cholesterol and phospholipid determinations.

FFA and TG were evaluated from the quantitative gas–liquid chromatographic analysis of their fatty acyl methyl esters performed with an arachidic acid internal standard. The quantitative gas-liquid chromatographic analysis of fatty acid methyl esters was also used for the determination of esterified cholesterol and phospholipid fractions in plasma membranes, because the quantities were too small for an accurate colorimetric measurement. Quantities of TG, FFA, phospholipids, and esterified cholesterol as low as 0.8 μg could be effectively determined by quantitative gas–liquid chromatographic analysis of their fatty acid methyl esters. Equivalent results by both methods were obtained in a few experiments when the phospholipids and cholesteryl esters were determined both colorimetrically and from the quantitative gas–liquid chromatographic analysis of their fatty methyl esters.

GEDE were submitted to reductive hydrolysis with 62% sodium bis-(2-methoxyethoxy) aluminum hydride in toluene (31) and the resulting glycerol ethers were quantitatively assayed as isopropylidene derivatives by gas–liquid chromatography using a known amount of heptadecyl glycerol as an internal standard. The values of alkyl glycerols were then multiplied by 2.5 to obtain the amounts of intact GEDE molecules (molecular weight ~832).

Gas–liquid chromatography

Fatty acid methyl esters and dimethyl acetals of aldehydogenic chains were obtained by refluxing the lipid samples with 5 ml of 5% sulfuric acid in methanol–benzene 25:1 and were analyzed isothermally at 185°C with 6 ft × ⅛ in. (i.d.) glass columns packed with 10% EGSS-X on 100–120 mesh Gas Chrom P (Applied Science) using a Barber and Coleman 5000 or a Perkin Elmer 3920 gas chromatograph equipped with hydrogen flame detectors. The peaks were identified by comparison with the retention time of pure standards of fatty acid methyl esters purchased from Applied Science Laboratories and Supelco (Bellefonte, PA) chromatographed under the same conditions. Quantitative response agreed with the stated composition of the standard mixtures NHI-D and NHI-F from Supelco. Peak areas in the gas chromatograms were calculated by triangulation.

Glycerol ethers were analyzed as isopropylidene derivatives at 195°C on the same columns used for the analysis of fatty acid methyl esters. The amount of total glycerol ethers was evaluated from comparison of their peak areas with the internal standard peak area. A known mixture of 15:0, 16:0, and 18:0 glycerol ethers was used to ascertain the quantitative response of the apparatus.

Statistical tests

Statistical significance of the differences between Balb/c3T3 and SV3T3 or SV3T3 Rev cells, between the whole cells and plasma membranes of each line, and between the whole cells and FCS was determined using the Wilcoxon test when the number of experiments was high enough to permit its applicability; 0.05 was considered the minimum level of significance.

RESULTS

Lipid composition of whole cells and plasma membrane fractions from Balb/c3T3, SV3T3, and SV3T3 Rev cells

As shown in **Table 1,** the lipids of Balb/c3T3 cells were mainly composed of phospholipids and free cholesterol; the amounts were similar to those found in other untransformed cultured cells, e.g., L cells (32), human diploid fibroblasts (8), BHK-21 cells (33), hamster embryonic fibroblasts (11), and Swiss 3T3 cells (12). The 3T3 cells also contained minor amounts of FFA and TG, similar to those found in L cells (32) and Swiss 3T3 cells (12). GEDE were present in 3T3 cells in the same amounts as in LM cells (34).

Plasma membrane fractions of 3T3 cells, prepared by the method of Brunette and Till (22) after mild trypsinization of the cells revealed a 3-fold enrichment of cholesterol and a nearly 2-fold increase of phospholipids with respect to the whole cells. These differences account for the higher cholesterol to phospholipid molar ratio in 3T3 plasma membranes (~0.600) in comparison to that in whole cells (~0.360). Our value for cholesterol to phospholipid molar ratio of 3T3 plasma membrane fractions is reasonably comparable to that previously reported for plasma membranes of chick embryo fibroblasts (14), L cells grown either in suspension (32) or as monolayers (35), and BHK-21 cells whether grown in suspension (36) or as monolayers (33). By contrast, our values for free cholesterol and phospholipid contents of plasma membrane fractions from untransformed 3T3 cells are lower than those reported for plasma membranes of chick embryo fibroblasts (14). Differences in the cell lines used, or in the procedure used for preparation of the plasma membrane fractions account for these differences. Moreover, 3T3 cell plasma membrane fractions did not contain TG and esterified cholesterol

TABLE 1. Lipid content of whole cells and plasma membrane fractions of Balb/c3T3, SV3T3, and SV3T3 Rev cells[a]

Lipid Class	Whole Cells						Plasma Membranes					
	Balb/c3T3		SV3T3		SV3T3 Rev		Balb/c3T3		SV3T3		SV3T3 Rev	
Total PL	165.0 ± 7.4	(18)	129.0 ± 4.1[b]	(21)	164.9 ± 7.7	(19)	303.0 ± 7.8	(3)	246.0 ± 30.5	(7)	211.8 ± 13.3	(4)
Total CL	28.4 ± 1.6	(18)	25.3 ± 1.6	(21)	28.5 ± 1.7	(19)	90.0 ± 7.5	(3)	105.0 ± 12.6	(7)	82.8 ± 7.5	(4)
CL/PL molar ratio	0.359 ± 0.026	(18)	0.390 ± 0.026	(21)	0.340 ± 0.017	(19)	0.606 ± 0.08	(3)	0.923 ± 0.148	(7)	0.633 ± 0.055	(4)
Esterified CL[c]	2.8 ± 0.8	(11)	5.7 ± 1.6	(8)	3.2 ± 0.7	(9)	ND[d]	(2)	ND[d]	(1)	25.8 ± 5.1	(2)
FFA	5.5 ± 1.2	(13)	5.1 ± 1.3	(13)	4.1 ± 0.5	(19)	23.0	(1)	36.6	(1)	40.7 ± 16.3	(2)
TG[e]	3.6 ± 0.7	(11)	3.7 ± 0.5	(11)	4.7 ± 0.6	(15)	ND[d]	(1)	14.0	(1)	ND[d]	(2)
GEDE	1.4 ± 0.4	(4)	1.7 ± 0.1	(2)	1.9 ± 0.5	(4)	NC[f]		NC[f]		NC[f]	

[a] Values, expressed as μg per mg protein, are the means ± SEM of a number of separate preparations listed in parentheses.
[b] Significantly different from untransformed Balb/c3T3 cells at $P < 0.01$.
[c] Esterified cholesterol in whole cells was assayed colorimetrically; the content of esterified cholesterol in plasma membranes was calculated from the molar content of the fatty acid methyl esters in cholesterol esters (see Materials and Methods).
[d] ND, not detectable under the analytical conditions used.
[e] The amounts of TG were calculated according to the formula (mol TG fatty acids/3) × molecular weight of TG. The moles of fatty acids were determined by quantitative gas–liquid chromatography as described in Materials and Methods; based on fatty acid composition, the molecular weights of whole cell and plasma membrane TG were 833 and 845, respectively.
[f] NC, not calculated. Although detected in the thin-layer chromatograms of plasma membrane neutral lipids, the GEDE were too low for an accurate determination.

but revealed amounts of FFA much higher than those found in whole cells; this latter result was also observed in the case of L cells (32).

In comparison to the untransformed 3T3 cells, whole SV3T3 cells showed a significantly lower phospholipid content and almost the same content of FFA, TG, and GEDE; the lower content of total cholesterol and the increase of esterified cholesterol were not significant. In plasma membrane fractions from SV3T3 cells there was an enrichment of cholesterol and phospholipids rather similar to that observed in the 3T3 cell plasma membrane fractions. However, the increase of the cholesterol to phospholipid molar ratio of SV3T3 cell plasma membrane fractions was not significant. Only free cholesterol was present in plasma membrane fraction of SV3T3 cells together with appreciable amounts of FFA as well as TG.

The whole SV3T3 Rev cells had the same content of total phospholipids and cholesterol as the untransformed 3T3 cells; the content of esterified cholesterol, FFA, TG, and GEDE was also similar to that found in the 3T3 cells. Plasma membrane fractions of SV3T3 Rev cells contained both free and esterified cholesterol, rather high amounts of FFA, and no detectable TG. Differences in the content of cholesterol and phospholipids of SV3T3 Rev cells plasma membrane fractions, when compared with 3T3 cell plasma membrane fractions, were not significant.

The phospholipid composition of whole cells and plasma membrane fractions of 3T3, SV3T3, and SV3T3 Rev cells are shown in **Table 2.** The phospholipids of whole 3T3 cells were mainly composed of PC and PE followed by SP and the combined PI and PS fraction, a pattern also observed in several untransformed cultured cells (8, 10, 12, 16, 32, 33). The phospholipid composition of plasma membrane fractions isolated from 3T3 cells was characterized by the absence of DPG and by a proportion of SP three times higher than that found in the whole cells. Both of these characteristics have been considered as indicating the purity of plasma membrane preparations (32, 37). In addition, plasma membrane fractions of 3T3 cells in comparison to whole cells showed an enrichment of PI + PS.

The whole SV3T3 as well as SV3T3 Rev cells had a phospholipid pattern comparable to that found in the untransformed 3T3 cells; only the SV3T3 Rev cells showed a significant increase of the SP proportion. In comparison to the whole cells of each respective line, the plasma membrane fractions isolated from SV3T3 and SV3T3 Rev cells contained a higher proportion of SP and were free of DPG, as was the case of plasma membrane fractions of 3T3 cells.

Fatty acid composition of lipid classes from whole cells and isolated plasma membrane fractions

Table 3 shows the fatty acid composition of the major glycerophospholipid classes of whole 3T3, SV3T3, and SV3T3 Rev cells. In PE of whole 3T3 cells, there was a prevalence of stearic and arachidonic acids over palmitic and oleic acids and noticeable proportions of arachidonic and C_{22} polyunsaturated fatty acids. PC of whole 3T3 cells had palmitic and oleic acids as major fatty acids followed by stearic and palmitoleic acids; arachidonic and C_{22} polyunsaturated acids were contained at a lower level than in PE. The fatty acids of the combined PI and PS fraction of 3T3 cells were predominantly stearic and

TABLE 2. Phospholipid composition of whole cells and plasma membrane fractions of Balb/c 3T3, SV3T3, and SV3T3 Rev cells[a]

Phospholipid	Whole Cells[b]			Plasma Membranes[c]		
	Balb/c3T3 (5)	SV3T3 (9)	SV3T3 Rev (10)	Balb/c3T3 (2)	SV3T3 (3)	SV3T3 Rev (3)
DPG	2.9 ± 0.9	3.0 ± 0.3	1.7 ± 0.3	ND[e]	ND[e]	ND[e]
PE	26.6 ± 1.3	22.1 ± 1.7	25.0 ± 0.6	17.4 ± 10.1	24.9 ± 1.2	20.5 ± 3.0
PC	48.6 ± 2.3	49.8 ± 1.5	47.6 ± 1.4	38.1 ± 6.3	41.8 ± 4.7	39.4 ± 4.5
PI + PS	13.0 ± 1.6	13.5 ± 0.8	12.6 ± 1.0	22.5 ± 0.1	15.5 ± 3.9	17.9 ± 2.8
SP	7.8 ± 0.8	10.7 ± 0.9	11.0 ± 0.8[d]	22.1 ± 3.7	17.9 ± 2.9	22.1 ± 5.1
PA	0.7 ± 0.7	0.9 ± 0.7	0.0 ± 0.5	ND[e]	ND[e]	ND[e]
LPC	0.4 ± 0.4	ND[e]	1.1 ± 0.5	ND[e]	ND[e]	ND[e]

[a] Data, expressed as mole percentage, are the means ± SEM of a number of separate preparations listed in parentheses.

[b] The whole cell phospholipids were separated by two-dimensional thin-layer chromatography and analyzed for phosphorus content after acid digestion.

[c] The composition of the phospholipid classes of plasma membranes was evaluated from the quantitative determination of their fatty acid methyl esters (see Materials and Methods) after taking into account the different fatty acid mole contribution in sphingomyelin.

[d] Significantly different from Balb/c3T3 cells at $P < 0.05$.

[e] ND, not detectable when plates, which had been spotted with 100–200 μg total phospholipids were sprayed with molybdenum blue reagent (27) after two-dimensional thin-layer chromatography.

arachidonic acids followed by oleic acid, while palmitic, palmitoleic and C_{22} polyunsaturated acids were minor components. This trend is similar to that found in glycerophospholipid classes of normal tissues of different animal species (38). Linoleic acid was a minor component in PE, PC, and PI + PS while the n-9 and n-6 isomers of eicosatrienoic acid (the former more prevalent than the latter) constituted a small percentage of the total fatty acids in PE, PC, and PI + PS of 3T3 cells. In addition, appreciable quantities of C_{16} and C_{18} alkenyl side chains were detected as dimethylacetals in PE of 3T3 cells, while

TABLE 3. Fatty acid composition of phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol + phosphatidylserine from whole Balb/c3T3, SV3T3, and SV3T3 Rev cells[a]

Fatty Acid[b]	Phosphatidylethanolamine			Phosphatidylcholine			Phosphatidylinositol + Phosphatidylserine		
	Balb/c3T3 (16)	SV3T3 (9)	SV3T3 Rev (17)	Balb/c3T3 (15)	SV3T3 (12)	SV3T3 Rev (16)	Balb/c3T3 (8)	SV3T3 (7)	SV3T3 Rev (8)
14:0	0.6 ± 0.2	1.1 ± 0.4	0.9 ± 0.2	3.3 ± 0.4	3.1 ± 0.3	2.2 ± 0.2[e]	0.6 ± 0.2	0.8 ± 0.2	0.2 ± 0.1
16:0 DMC[c]	2.7 ± 0.5	3.6 ± 0.9	4.0 ± 0.8	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1			
16:0	9.2 ± 1.1	13.0 ± 1.5[e]	10.3 ± 1.1	31.7 ± 1.5	32.8 ± 0.8	34.7 ± 1.2	4.1 ± 0.6	8.3 ± 1.3[f]	4.9 ± 0.4
16:1	2.0 ± 0.4	4.6 ± 0.6[f]	4.6 ± 0.4[f]	8.0 ± 0.7	10.9 ± 0.8[e]	13.1 ± 0.6[f]	2.5 ± 0.8	2.5 ± 0.4	2.9 ± 0.4
18:0 DMC[c]	1.7 ± 0.4	1.1 ± 0.3	1.9 ± 0.5		0.2 ± 0.1	0.3 ± 0.1			
18:0	20.5 ± 0.8	18.2 ± 2.3	19.6 ± 1.0	12.6 ± 1.2	10.5 ± 1.0	7.5 ± 0.4[f]	46.8 ± 2.6	39.1 ± 1.3[f]	44.7 ± 3.5
18:1[d]	14.9 ± 1.3	29.1 ± 1.4[f]	29.4 ± 1.4[f]	24.0 ± 1.5	31.8 ± 1.2[e]	34.6 ± 1.0[f]	15.2 ± 1.1	22.1 ± 2.1[f]	25.9 ± 2.0[f]
18:2	0.8 ± 0.2	1.8 ± 0.3[f]	0.9 ± 0.2	1.5 ± 0.2	1.8 ± 0.2	1.2 ± 0.2	1.5 ± 0.7	1.3 ± 0.3	0.4 ± 0.1
20:3 (n-9)	2.8 ± 0.6	1.0 ± 0.3	3.1 ± 0.6	0.8 ± 0.2	0.3 ± 0.1	0.6 ± 0.2	3.1 ± 0.9	2.6 ± 0.7	6.0 ± 1.2
20:3 (n-6)	0.6 ± 0.1	1.1 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1[f]	0.9 ± 0.3	1.1 ± 0.3	0.5 ± 0.2
20:4	22.3 ± 1.4	11.0 ± 1.2[f]	12.2 ± 0.9[f]	10.5 ± 1.3	4.6 ± 0.6[f]	2.8 ± 0.5[f]	18.3 ± 3.8	12.5 ± 1.3	8.6 ± 1.2[e]
20:5	4.0 ± 0.5	1.9 ± 0.4[f]	1.7 ± 0.3[f]	1.9 ± 0.3	0.7 ± 0.2[e]	0.5 ± 0.1[f]	0.7 ± 0.3	1.5 ± 0.3	0.6 ± 0.2
22:2	0.1 ± 0.1		0.3 ± 0.2						
22:4	3.8 ± 0.5	1.9 ± 0.3[e]	1.8 ± 0.3[f]	0.7 ± 0.2	0.7 ± 0.2	0.3 ± 0.1[f]	1.3 ± 0.5	0.8 ± 0.2	0.4 ± 0.1
22:5	7.2 ± 0.9	5.8 ± 0.9	4.8 ± 0.5	2.0 ± 0.4	1.1 ± 0.2	0.7 ± 0.2[f]	2.7 ± 0.7	3.6 ± 0.7	2.9 ± 0.6
22:6	6.8 ± 0.6	4.8 ± 0.7	4.2 ± 0.5	2.5 ± 0.6	1.0 ± 0.2[e]	1.0 ± 0.3[f]	2.4 ± 0.7	3.8 ± 0.9	2.2 ± 0.5

[a] All values, expressed as weight percentage of the total fatty acids, are the means ± SEM of a number of separate experiments listed in parentheses.

[b] Fatty acids are designated by chain length: number of double bonds.

[c] DMC are dimethyl acetals from alkenyl side chains of plasmalogens.

[d] Although the location of the double bond was not determined, octadecenoic acid was reported throughout the text under the name of the more common isomer, oleic acid.

[e] Significantly different from Balb/c3T3 cells at $P < 0.05$.

[f] Significantly different from Balb/c3T3 cells at $P < 0.01$.

their proportion in PC was very low, indicating that certain proportions of PE and PC in 3T3 cells were composed of alkenyl species.

The characteristic fatty acid patterns in PE, PC, and PI + PS observed in whole untransformed 3T3 cells were also present in SV3T3 and SV3T3 Rev cells. However, in comparison to the untransformed 3T3 cells, both the SV3T3 cells and SV3T3 Rev cells showed a remarkable increase of oleic and palmitoleic acids concomitant with a reduction of arachidonic, $C_{20:5}$, and $C_{22:4}$ acids in PE. The amount of palmitic acid in the PE fraction was slightly higher in SV3T3 cells than in 3T3 cells. The proportion of C_{16} and C_{18} alkenyl side chains in PE of SV3T3 and SV3T3 Rev cells was almost the same as in PE of untransformed 3T3 cells. Even in the PC of SV3T3 cells, as well as SV3T3 Rev cells, there was a significant increase in the proportion of oleic and palmitoleic acids, less marked than in the PE and associated with a decrease of arachidonic, $C_{20:5}$, and $C_{22:6}$ acids; an increase of oleic acid was also found in the combined PI and PS fraction of both SV3T3 and SV3T3 Rev cells but was accompanied by a significant decrease of arachidonic acid only in SV3T3 Rev cells.

As shown in **Table 4,** the fatty acid profiles of PE, PC, and PI + PS of plasma membrane fractions isolated from 3T3 cells were similar to those found in whole 3T3 cells with the exception that, in PC of plasma membranes compared with whole cells, there was a significantly higher level of palmitic acid counterbalanced by a lower proportion of arachidonic and C_{22} polyunsaturated acids. Plasma membrane fractions isolated from either SV3T3 or SV3T3 Rev cells exhibited the same increase in the proportion of oleic and palmitoleic acids, and an equivalent decrease of arachidonic acid in PE as observed on comparing the whole cells of the respective line with the untransformed counterpart. An increase of oleic and palmitoleic acids was also found in the PC of plasma membrane fractions from SV3T3 and SV3T3 Rev cells, while an evident decrease of arachidonic acid was present only in the PC of SV3T3 Rev cells plasma membrane fractions. The proportion of PC palmitic acid was lower in the plasma membranes of SV3T3 and SV3T3 Rev cells than in those of 3T3 cells. An increase of oleic acid associated with a decrease of arachidonic acid was noted in the PI + PS fraction of plasma membrane fractions of SV3T3 Rev cells, but not in those isolated from SV3T3 cells.

Table 5 shows the fatty acid composition of SP of whole cells and plasma membrane fractions of 3T3, SV3T3, and SV3T3 Rev cells. The SP isolated from whole 3T3 cells and plasma membrane fractions exhibited a characteristic pattern due to the remarkably high level of palmitic acid and noticeable proportions of 22:0 (behenic), 22:1 (erucic), 23:0, 24:0 (lignoceric), and 24:1 (nervonic) acids. No significant variations of this pattern were found in SV3T3 and SV3T3 Rev cells, either at the level of whole cells or in the plasma membrane fractions.

No significant differences were found in the fatty acid composition of cholesteryl esters, TG, and FFA of 3T3, SV3T3, and SV3T3 Rev cells (data not shown). High percentages of palmitic, oleic, and palmitoleic acids were characteristic of cholesteryl esters, while TG had palmitic and oleic followed by stearic as

TABLE 4. Fatty acid composition of phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol + phosphatidylserine from plasma membrane fractions of Balb/c3T3, SV3T3, and SV3T3 Rev cells[a]

	Phosphatidylethanolamine			Phosphatidylcholine			Phosphatidylinositol + Phosphatidylserine		
Fatty Acid[b]	Balb/c3T3 (2)	SV3T3 (3)	SV3T3 Rev (3)	Balb/c3T3 (2)	SV3T3 (3)	SV3T3 Rev (3)	Balb/c3T3 (2)	SV3T3 (3)	SV3T3 Rev (3)
14:0	3.1 ± 1.0	2.1 ± 0.3	0.7 ± 0.4	5.6 ± 0.8	2.6 ± 0.5	3.1 ± 0.7		1.1 ± 0.6	0.6 ± 0.6
16:0 DMA[c]	3.7 ± 3.7	4.9 ± 0.4	3.0 ± 2.1						
16:0	12.2 ± 4.5	14.2 ± 3.0	18.8 ± 5.3	55.9 ± 8.0	35.8 ± 0.2	46.4 ± 5.2	5.5 ± 1.8	13.6 ± 1.5	7.7 ± 2.0
16:1	2.9 ± 0.1	5.1 ± 0.3	5.6 ± 0.6	4.1 ± 1.6	8.0 ± 1.0	11.4 ± 1.4	3.2 ± 2.2	3.0 ± 1.4	5.0 ± 1.7
18:0 DMA[c]	1.1 ± 1.1	1.4 ± 1.0			0.4 ± 0.2				
18:0	22.9 ± 12.6	17.4 ± 3.9	18.6 ± 0.8	10.2 ± 1.4	10.6 ± 0.6	8.0 ± 1.1	50.9 ± 3.8	34.1 ± 3.3	45.9 ± 3.9
18:1[d]	11.2 ± 3.4	23.1 ± 3.7	27.5 ± 0.7	17.2 ± 4.2	30.6 ± 2.1	27.1 ± 3.5	17.3 ± 3.6	18.0 ± 3.6	22.8 ± 1.2
18:2	0.2 ± 0.2	0.9 ± 0.5	0.7 ± 0.6	0.5 ± 0.5	1.7 ± 0.2	0.9 ± 0.7	0.3 ± 0.3	0.8 ± 0.4	0.9 ± 0.5
20:3 (n-9)	0.4 ± 0.4	1.0 ± 0.5	2.6 ± 0.7	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.5	0.5 ± 0.2	2.2 ± 0.7	3.0 ± 1.1
20:3 (n-6)	0.3 ± 0.3	0.5 ± 0.3		0.3 ± 0.3	0.7 ± 0.2		0.7 ± 0.5	1.5 ± 0.5	
20:4	21.3 ± 3.1	11.6 ± 1.8	10.4 ± 1.3	4.0 ± 0.2	4.3 ± 1.1	1.7 ± 0.5	9.3 ± 2.1	13.2 ± 2.6	6.8 ± 1.1
20:5	3.0 ± 0.2	1.9 ± 0.2	2.4 ± 0.5	0.4 ± 0.4	0.5 ± 0.1	0.5 ± 0.3	0.8 ± 0.2	1.1 ± 0.6	0.6 ± 0.3
22:2	0.3 ± 0.3	0.3 ± 0.1	0.7 ± 0.4						
22:4	4.7 ± 1.9	3.4 ± 0.5	1.5 ± 0.6	0.4 ± 0.4	0.8 ± 0.4	0.1 ± 0.1	2.5 ± 1.3	2.9 ± 1.6	1.0 ± 0.6
22:5	6.9 ± 5.7	6.7 ± 0.9	4.0 ± 0.9	0.5 ± 0.5	1.7 ± 0.6	0.1 ± 0.1	4.5 ± 3.3	4.1 ± 2.0	3.2 ± 0.4
22:6	5.8 ± 4.6	5.5 ± 0.9	3.5 ± 1.0	0.5 ± 0.5	1.8 ± .09	0.2 ± 0.2	4.6 ± 3.9	4.4 ± 2.7	2.4 ± 0.4

Footnotes as in Table 3.

TABLE 5. Fatty acid composition of sphingomyelin from whole Balb/c3T3, SV3T3, and SV3T3 Rev cells and their plasma membrane fractions[a]

Fatty Acid[b]	Whole Cells			Plasma Membranes		
	Balb/c3T3 (9)	SV3T3 (9)	SV3T3 Rev (10)	Balb/c3T3 (2)	SV3T3 (3)	SV3T3 Rev (3)
14:0	1.3 ± 0.3	2.3 ± 0.3[d]	2.0 ± 0.9	0.7 ± 0.8	1.3 ± 0.7	2.1 ± 1.3
16:0	50.8 ± 4.4	47.9 ± 2.4	60.3 ± 3.3[d]	37.3 ± 15.4	50.7 ± 2.9	51.1 ± 8.4
16:1	0.8 ± 0.3	1.4 ± 0.5	1.5 ± 0.4	0.5 ± 0.5	4.7 ± 1.9	4.6 ± 2.1
18:0	8.8 ± 0.4	7.0 ± 0.7[d]	5.4 ± 0.5[e]	15.8 ± 1.1	9.4 ± 2.4	7.3 ± 0.9
18:1[c]	6.6 ± 1.8	7.0 ± 1.3	6.5 ± 1.1	8.6 ± 4.9	6.6 ± 3.7	14.8 ± 5.6
18:2	0.1 ± 0.1	0.6 ± 0.4	0.4 ± 0.2		0.7 ± 0.4	
20:0	0.9 ± 0.3	1.3 ± 0.9	0.4 ± 0.1			
20:1				1.7 ± 1.2		2.5 ± 2.5
22:0	3.9 ± 0.8	4.2 ± 0.6	3.4 ± 0.4	5.0 ± 1.6	4.4 ± 0.9	3.0 ± 0.3
22:1	1.9 ± 0.7	1.0 ± 0.3	0.7 ± 0.3	0.7 ± 0.1	0.6 ± 0.4	0.9 ± 0.7
23:0	1.2 ± 0.4	2.9 ± 0.9[d]	1.4 ± 0.8	3.6 ± 1.8	1.6 ± 0.4	0.7 ± 0.7
23:1	0.5 ± 0.3	0.4 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	0.3 ± 0.3	
24:0	8.7 ± 1.9	11.9 ± 2.2	7.1 ± 0.7	10.8 ± 3.9	7.0 ± 2.0	5.1 ± 0.8
24:1	14.5 ± 3.1	12.1 ± 1.2	10.6 ± 0.9	15.2 ± 1.4	12.7 ± 3.9	7.7 ± 2.1

[a,b] As in Table 3.
[c] See note *d* in Table 3.
[d] Significantly different from Balb/c3T3 cells at $P < 0.05$.
[e] Significantly different from Balb/c3T3 cells at $P < 0.01$.

major fatty acids; FFA were mainly composed of palmitic and stearic acids followed by oleic acid. Linoleic and arachidonic acids were minor fatty acids in cellular cholesteryl esters, TG, and FFA. In addition, the analyses of FFA plasma membrane fractions did not reveal differences between 3T3 and SV3T3 or SV3T3 Rev cells. The TG isolated from SV3T3 cell plasma membranes showed the same fatty acid pattern as that found in whole cells, while the cholesteryl esters in SV3T3 Rev cell plasma membranes showed a higher proportion of palmitoleic acid and a lower level of oleic acid than the cholesteryl esters of whole SV3T3 Rev cells (data not shown).

Lipid composition of FCS

FCS used throughout this study contained 300 μg of phospholipids, 18 μg of FFA, 9 μg of TG, and 350 μg of cholesterol, mainly as esterified cholesterol, per ml. FCS phospholipids were composed of 48.3% PC, 23.2% LPC, 26.2% SP, and 2.1% PI + PS.

The fatty acid compositions of the individual lipid classes of FCS are reported in **Table 6.** Cholesteryl esters contained high proportions of palmitic and oleic acids, and noticeable amounts of palmitoleic, linoleic, and arachidonic acids; myristic and stearic acids were minor components. In comparison to cellular cholesteryl esters, the serum cholesteryl esters contained significantly higher percentages of palmitic, linoleic, and arachidonic acids, and lower levels of palmitoleic and stearic acids. The FFA of FCS were mainly composed of palmitic and oleic followed by stearic acids. In comparison to the cellular FFA, the serum FFA contained a much higher proportion of oleic acid. The major fatty acids of FCS TG were palmitic and oleic acids followed by stearic acid, a pattern similar to that found in cellular TG. In the FCS, PC and LPC had palmitic, stearic, and oleic acids in almost equal proportions as major fatty acids and also contained appreciable amounts of arachidonic and C_{22} polyunsaturated acids. Trace amounts of C_{16} and C_{18} alkenyl chains, not reported in the table, were also present in PC of FCS. This profile does not coincide with that found in cellular PC where, regardless of the cell line, there was a net prevalence of palmitic over stearic acids and a higher proportion of palmitoleic acid in comparison to that present in the PC of FCS. The fatty acid composition of SP from FCS showed the same characteristics as those found in the cellular SP, except for the significantly lower proportion of palmitic acid and the much higher level of behenic acid. Small amounts of eicosatrienoic acid, mainly as the n-6 isomer, were in all lipid classes of FCS.

In conclusion, the lipid analysis of FCS revealed that, despite some similarities, the acyl chain profiles of the cellular lipids only partially reflect those found in the serum. This finding suggests that, although cultured cells have been shown to incorporate lipids from growth media (18–20), 3T3, SV3T3, and SV3T3 Rev cells exhibit a certain capacity to selectively utilize serum lipids and/or remodel acyl chain make-up.

DISCUSSION

The cellular system used in the present study is a widely studied in vitro model for cellular changes

TABLE 6. Fatty acid composition of the lipid classes from fetal calf serum[a]

Fatty Acids[b]	Cholesteryl Esters (6)	FFA (6)	TG (6)	PC (5)	LPC (4)	SP (5)
14:0	2.2 ± 0.3	3.5 ± 0.9	5.2 ± 0.3	0.5 ± 0.2	0.6 ± 0.4	0.5 ± 0.2
16:0	29.7 ± 1.7	27.8 ± 2.5	42.0 ± 4.7	24.3 ± 1.4	30.9 ± 2.3	34.6 ± 1.6
16:1	10.5 ± 1.1	4.3 ± 0.7	9.1 ± 0.6	1.2 ± 0.4	2.5 ± 0.8	0.4 ± 0.3
18:0	3.2 ± 0.3	17.9 ± 1.1	10.3 ± 2.1	24.7 ± 0.8	24.2 ± 2.5	10.3 ± 0.4
18:1[c]	35.6 ± 0.9	25.2 ± 0.7	24.7 ± 4.3	25.9 ± 1.0	24.2 ± 2.0	2.1 ± 0.3
18:2	8.8 ± 0.4	3.7 ± 0.2	2.4 ± 0.9	1.9 ± 0.1	2.8 ± 0.2	
18:3	0.9 ± 0.3	0.1 ± 0.1	0.9 ± 0.4	0.9 ± 0.2		
20:0						2.8 ± 0.1
20:3 (n-9)	0.1 ± 0.1	0.8 ± 0.3	0.3 ± 0.3	0.2 ± 0.0	0.1 ± 0.1	
20:3 (n-6)	0.8 ± 0.2	1.5 ± 0.4	0.2 ± 0.1	3.3 ± 0.2	1.8 ± 0.4	
20:4	7.2 ± 0.6	8.5 ± 1.1	2.7 ± 1.5	5.6 ± 0.2	7.5 ± 2.0	
20:5	0.8 ± 0.4	0.7 ± 0.4	0.5 ± 0.3	0.3 ± 0.2	0.5 ± 0.4	
22:0						14.6 ± 0.3
22:1						1.7 ± 0.2
22:2		1.3 ± 0.6	0.4 ± 0.4			
22:4				0.9 ± 0.4	0.2 ± 0.2	
22:5		2.0 ± 0.7	1.0 ± 0.5	5.8 ± 1.0	2.7 ± 1.3	
22:6	0.2 ± 0.2	2.7 ± 0.9	0.3 ± 0.3	4.5 ± 0.8	2.0 ± 0.9	
23:0						2.9 ± 0.8
23:1						2.1 ± 0.8
24:0						9.8 ± 0.3
24:1						18.2 ± 1.7

Values, expressed as weight percentage of the total fatty acids, are the means ± SEM from the number of fetal calf serum batches indicated in parentheses.

Footnotes *a*, *b*, and *c* as in Table 3.

See note *d* in Table 3.

associated with malignant transformation. Untransformed Balb/c3T3 cells are contact-inhibited (39) and not tumorigenic (40) unless injected adhering to glass beads (41). SV40-transformed cells of the same line (SV3T3) show several biological and biochemical changes (42–44) shared by tumor cells growing in vivo. The concanavalin A-selected revertant cells (SV3T3 Rev) exhibit density-dependent growth, characteristic of the untransformed parental cells, although containing SV40 genetic information (17).

In comparison to untransformed 3T3 whole cells, the SV3T3 cells showed a significantly lower phospholipid content, a difference also observed in other virus-transformed cell systems (8, 11) and in different neoplasms (45–49). It is noteworthy that the phospholipid content of the SV3T3 Rev whole cells returns to the level characteristic of untransformed 3T3 cells. However, the phospholipid content of plasma membrane fractions from either SV3T3 or SV3T3 Rev cells was not significantly different from that found in 3T3 cell plasma membranes, so that the significant differences in whole cell phospholipid content may reflect changes in internal cellular membranes or protein content.

No significant differences in the cholesterol to phospholipid molar ratios of 3T3, SV3T3, and SV3T3 Rev cells were observed at the level of both whole cells and plasma membrane fractions. An unchanged cholesterol to phospholipid molar ratio was also found in the plasma membranes of cultured chick embryo fibroblasts, after transformation by RNA viruses (14). On the other hand, the cholesterol to phospholipid molar ratio was reduced in mouse leukemic lymphocytes (50) but was increased in the plasma membranes of various rat and mouse hepatomas (37) compared to the normal counterparts. All these results suggest that changes in cholesterol content or cholesterol to phospholipid ratio when present in malignant cells may represent cell-specific differences, contradicting the generalization that cholesterol level is relevant in the control of cell growth (51, 52). In whole SV3T3 cells the level of GEDE was practically the same as in untransformed 3T3 cells. In a number of cultured cells, both normal and transformed, Howard, Butler, and Bailey (9) found a high level of ether-linked lipids, which they correlated with the decreased activity of α-glycerophosphate dehydrogenase in these cells.

The more evident alteration in the lipid composition of virally transformed 3T3 cells was mainly confined to the phospholipid acyl chains. The major changes consisted in an increase of palmitoleic and oleic acids and a decrease of arachidonic acid which affected the PE of SV3T3 cells at the level of whole cells and of plasma membranes. Similar changes were also observed in the acyl chain profile of PC from

whole SV3T3 cells, although to a lesser extent than in PE, while in PC of SV3T3 plasma membranes the increase of monoenoic acids was not associated with a significant variation of arachidonic acid. An increase of oleic acid was also present in the SV3T3 PI + PS at the level of whole cells but not of plasma membranes.

A lower proportion of arachidonic acid concomitant with an increased content of oleic acid was also found in total phospholipids of SV40-transformed WI-38 and Swiss 3T3 cells (9, 13), and in RSV-transformed chick embryo fibroblasts at the level of whole cell phospholipid fractions and of plasma membrane total phospholipids (15). By using a temperature-sensitive mutant of RSV, the differences in acyl group composition of RSV-transformed chick embryo fibroblasts were found to be related to viral gene function but occurred relatively late after switching to the permissive temperature, indicating that the changes in acyl group composition were not likely to be the primary cause of the early membrane changes associated with transformation (53). Moreover, comparison of normal and polyoma-transformed BHK cells did not reveal any significant difference in the acyl chain composition of individual phospholipid classes from plasma membranes (16).

It is worth noting that several neoplasms, whether primary or transplantable, solid or ascitic, fast- or slow-growing, when compared with their respective normal tissue, revealed changes in the acyl chain group composition of phospholipid classes (46, 48, 49, 54–57) which are consistent with the differences observed in the virally transformed cells, as shown in the present study and in other laboratories (9, 13, 15). Thus the increased level of monoenoic acids associated with the reduction of C_{20} and C_{22} polyunsaturated acids in phospholipids seems to be an abnormality associated to malignancy, independent of the transforming agent, type of cells, degree of differentiation, and conditions of microenvironment. In addition, the differences in the acyl chain composition observed in SV3T3 cells do not seem to be related to growth rate, since 3T3 and SV3T3 cells were both studied once they had reached maximum saturation density. Moreover, 3T3 cells when examined at their exponential phase of growth, exhibited the same percentages of palmitoleic, oleic, and arachidonic acids as the 3T3 cells at confluence (results not reported in tables). Furthermore, it should be mentioned that embryonic tissues at their maximum growth rate were shown to have a high level of polyunsaturated acids and a low percentage of monoenoic acids (58), a pattern which is the opposite of that found in neoplastic cells.

The finding that SV3T3 Rev cells, at variance with their reversion of other biochemical properties (17, 59), exhibited the same acyl chain changes as the SV3T3 cells indicates that an alteration in the acyl chain make-up of the surface phospholipids is a molecular event associated with viral genome integration, although not relevant in the loss of growth control. Nevertheless, it is quite possible that in SV3T3, as well as in SV3T3 Rev cells, the increase of oleic-containing phospholipid species may result, through their interaction with cholesterol (60), in a condensation of distinct regions of the lipid core of biological membranes, influencing some surface properties related to the structural arrangement of membrane lipids.

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