Lipid composition of Balb/c3T3, SV3T3, and Concanavalin A-selected revertant cells grown in media containing lipid-depleted serum

Donatella Tombaccini, Anna Fallani, Gabriele Mugnai, and Salvatore Ruggieri

Institute of General Pathology, University of Florence, 50134 Florence, Italy

Abstract The effects of growth in media supplemented with lipid-depleted fetal calf serum (LDS-media) on morphology, saturation density, and lipid composition were studied in Balb/c3T3, SV3T3, and Concanavalin A-selected SV3T3 revertant cells (SV3T3 Rev cells). Cells grown in media containing complete fetal calf serum (FCS-medium) or reconstituted FCS (RS-medium) were used as controls. Growth in LDS-media reduced saturation densities of both SV3T3 and SV3T3 Rev cells while it affected only slightly the saturation density of normal parental cells. Similar inhibitory effects on growth were also induced by exposure of RS-medium. Growth in LDS-medium did not change the typical morphology of the three cell lines. 3T3, SV3T3, and SV3T3 Rev cells grown in LDS-medium showed an accumulation of triacylglycerols and free fatty acids together with a reduction of free cholesterol. All these changes were also present, however, in cells grown in RS-medium. Growth in LDS-medium induced an increase of 16:1 and 18:1, a decrease of 20:4, and an accumulation of 20:3 (n-9) in phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol + phosphatidylserine of 3T3 cells. By contrast, only a slight accumulation of 20:3 (n-9) accompanied by a moderate increase of monoenoic acids was found in the phospholipids of SV3T3 cells grown in LDS-medium. SV3T3 Rev cells grown in LDS-medium showed changes in phospholipid fatty acid composition similar to those found in SV3T3 cells grown under the same conditions.—**Tombaccini, D., A. Fallani, G. Mugnai, and S. Ruggieri.** Lipid composition of Balb/c3T3, SV3T3, and Concanavalin A-selected revertant cells grown in media containing lipid-depleted serum. *J. Lipid Res.* 1981. **22:** 590–597.

Supplementary key words glycerophospholipids · fatty acid composition

The use of cell culture systems has promoted progress in the knowledge of lipid metabolism (1–3), of the role of lipids in cell growth (4–10), and of the relationship between lipid structure and cell surface properties (5, 11–16). In respect to animal tissues, cells grown in tissue culture offer the advantage of a more precise control of growth conditions and permit selective lipid alterations by using media of definite composition. Cell culture systems have proved to be even more useful for the study of lipid metabolism of malignant cells because of the availability of different lines of transformed cells which exhibit biological characteristics analogous to those of tumor cells in vivo (2), while avoiding interferences due to host–tumor relationships.

Previous studies have shown differences in phospholipid fatty acid profiles between SV40-transformed Balb/c3T3 cells (SV3T3 cells) and normal parental cells grown in a serum-containing medium (17). Altered fatty acid profiles did not revert to a normal pattern in Concanavalin A-selected SV3T3 revertant cells (SV3T3 Rev cells), a variant of SV3T3 cells with growth properties and molecular characteristics similar to those of untransformed Balb/c3T3 cells (18–20).

In this report, we studied the lipid composition of Balb/c3T3, SV3T3, and SV3T3 Rev cells grown in media supplemented with lipid-depleted serum in order to reveal possible differences in the lipid metabolism of these cells that would not otherwise be observed in the presence of lipids in the growth medium.

In view of the observation that a normal phenotype can be restored to transformed cells by decreasing the lipids available in the medium (8), saturation densities and morphology of 3T3, SV3T3, and SV3T3 Rev cells grown in the presence of lipid-depleted serum were also examined.

Abbreviations: Con A, Concanavalin A; PBS, phosphate-buffered saline; EGTA, ethylene-bis(oxyethylenenitrilo)tetraacetic acid; TG, triacylglycerols; FFA, free fatty acids; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine, PI, phosphatidylinositol; PS, phosphatidylserine; SP, sphingomyelin; LPC, lysolecithin; EFA, essential fatty acid; PUFA, polyunsaturated fatty acids; FCS, fetal calf serum; TLC, thin-layer chromatography.

MATERIALS AND METHODS

Cell lines

The Balb/c3T3 cells (clone A31 from Aaronson), SV3T3 cells (clone SVT2 from Aaronson), and SV3T3 Rev cells (clone 81 and 84, isolated by L. Culp (18)) were kindly supplied by Dr. P. H. Black (Microbiology Dept., Boston University Medical Center) and maintained in our laboratory as described in a previous paper (17).

Preparation of lipid-depleted serum and reconstituted serum

Commercial fetal calf serum (FCS) (Flow Lab., Irvine, Scotland and GIBCO, Grand Island, NY) was delipidated by multiple ethanol and diethyl ether extracts as described by Horwitz et al. (12). What we refer to as lipid-depleted serum is the delipidated dry residue of FCS solubilized by sonication in distilled water and subsequently adjusted to the original volume of FCS. Quantitative gas–liquid chromatographic analysis of methyl ester derivatives of the fatty acids released by saponification (0.6 N methanolic NaOH) showed that less than 1% of the total fatty acids present in the complete FCS remained in the lipid-depleted serum. The reconstituted serum was obtained by combining the delipidated dry residue of FCS with the extracted lipids in chloroform solution followed by evaporation of the solvent and sonication of the mixture in distilled water.

Growth in media containing complete, lipid-depleted or reconstituted fetal calf serum

Cells removed from tissue culture dishes by trypsinization were seeded in 75 cm^2 Falcon flasks at 1×10^6 cells (3T3 cells) or at 0.8×10^6 cells (SV3T3 and SV3T3 Rev cells) and grown in a medium constituted by Eagle's minimal essential medium with a four-fold concentration of vitamins and amino acids (MEM × 4) supplemented with 10% lipid-depleted FCS (LDS-medium). Cells grown in MEM × 4 supplemented with 10% complete FCS (FCS-medium) were used as controls. Cells were also grown in the presence of MEM × 4 supplemented with 10% reconstituted FCS (RS-medium) in order to evaluate whether manipulations other than the serum's lipid depletion could account for possible changes in the lipid composition, saturation density, and morphology of cells grown in LDS-medium.

In order to minimize selection effects that may occur when the cells are serially propagated in manipulated media (8), 3T3, SV3T3, and SV3T3 Rev cells were studied after only a single passage in LDS- or RS-media.

Regardless of the composition of growth media, cells were changed to a fresh medium the day after plating and then every other day. 3T3 cells grown in LDS- or RS-media reached their maximum saturation density on the 5th day after seeding, i.e., a day later than the cells grown in FCS-medium. SV3T3 cells grown in FCS-medium reached their maximum saturation density 7 days after seeding; when grown in LDS- or RS-media, they showed no further growth after 5 days, although cultures exhibited numerous empty spaces. SV3T3 Rev cells grown in LDS- or RS-media were confluent 5 days after seeding, i.e., a day later than when grown in FCS-medium. No increase of cell loss was evident during growth in LDS- or RS-media, as shown by microscopic examination of the cultures.

Cells were harvested using PBS containing 0.02% EGTA when they reached confluence or when they stopped growing. The detached cells were washed twice by centrifugation in PBS at 1500 rpm (International PR6000 centrifuge, rotor 269) and, after resuspension in 10 ml of PBS, they were sonicated with Labsonic sonicator, model 1510 (Braun, Melsungen, West Germany). Control of viability, routinely performed by trypan blue exclusion technique, showed that more than 80% of the cells were viable, regardless of the cell line and of the composition of the growth medium.

Lipid analysis

Total lipids were extracted from the sonicated cell suspensions according to Folch, Lees, and Sloane Stanley (21) and fractionated into neutral lipids and phospholipids by silicic acid column chromatography (Unisil 100–200 mesh, Clarkson Chemical Co. Williamsport, PA). Neutral lipids were then separated into sterol esters, triacylglycerols (TG), free fatty acids (FFA), and free sterols by thin-layer chromatography (TLC) on 0.25 mm silica gel H pre-coated plates (Merck, Darmstadt, West Germany) using hexane–diethyl ether–acetic acid 85:15:1. Phospholipids were fractionated into diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI) plus phosphatidylserine (PS), sphingomyelin (SP), and lysolecithin (LPC) by two-dimensional TLC 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.

Concentrations of TG, FFA, and phospholipid classes were determined by quantitative gas–liquid chromatographic analysis of their fatty acid methyl esters prepared by acid-catalyzed methanolysis (5% H_2SO_4 in methanol) using a known amount of

TABLE 1. Saturation densities of Balb/c3T3, SV3T3, and SV3T3 Rev cells grown in media containing 10% complete fetal calf serum (FCS-medium), lipid-depleted fetal calf serum (LDS-medium), or reconstituted fetal calf serum (RS-medium)[a]

Growth Media	Cell Line		
	Balb/c3T3	SV3T3	SV3T3 Rev[b]
FCS-medium	4.6 ± 0.3 (18)	18.5 ± 1.4 (18)	8.6 ± 0.5 (21)
LDS-medium	3.3 ± 0.3 (12)	5.3 ± 0.8 (9)	3.7 ± 0.5 (9)
RS-medium	3.0 ± 0.3 (4)	5.4 ± 1.6 (4)	3.4 ± 0.8 (5)

[a] Saturation densities are expressed as cells/cm^2 × 10^{-4} and are given as means ± SEM; number of experiments in parentheses.
[b] Variations between clones 81 and 84 of SV3T3 Rev cells were negligible and therefore means were calculated by combining data of the two clones.

arachidic acid as an internal standard. Analyses were performed at 185°C on glass columns (180 cm × 2 mm) packed with 10% EGSS-X on Gas Chrom P 100–120 mesh (Supelco, Bellefonte, PA) and mounted in a Perkin-Elmer gas chromatograph model 3920 equipped with hydrogen flame detectors. Identification of fatty acids was made by comparison with pure standards from Supelco. In the case of 20:3, the presence of n-9 and n-6 isomers was confirmed by the analysis of the ozonolysis products (22, 23) of the trienoic subfractions of fatty acid methyl esters isolated by argentation TLC (24).

Free sterols were assayed colorimetrically (25), whereas esterified sterols were submitted to saponification in 0.3 N ethanolic KOH and the extracted sterols were quantitatively analyzed as trimethylsilylether derivatives by gas–liquid chromatography with a coprostanol internal standard. Analyses were performed at 250°C on glass columns (180 cm × 2 mm) packed with 3% OV17 on Supelcoport 80–100 mesh (Supelco) using pure standards of cholesterol and desmosterol for peak identification.

Total phospholipids were determined by multiplying ×25 the phospholipid-phosphorus assayed on total lipid extracts following the method of Martin and Doty (26) after digestion with sulfuric acid–perchloric acid 3:2. Protein was determined on the sonicated cell suspensions following the method of Lowry et al. (27), with bovine serum albumin (Sigma) as a standard.

Statistical analysis

Student's *t* test was used to determine the statistical significance of differences between the following sets of growth conditions: *a*) between cells grown in LDS-medium and cells grown in FCS-medium; *b*) between cells grown in FCS-medium and cells grown in RS-medium; and *c*) between cells grown in LDS-medium and cells grown in RS-medium. Differences between 3T3 and SV3T3 or SV3T3 Rev cells under the same growth conditions were also submitted to statistical analysis. In the text only differences that are statistically significant have been discussed, with 0.05 being the minimum level of significance.

RESULTS AND DISCUSSION

Effect on saturation density and cell morphology

As shown in **Table 1**, SV3T3 cells grown in FCS-medium characteristically grew to a much higher saturation density than the untransformed parental cells. SV3T3 Rev cells showed an intermediate saturation density. Saturation density of 3T3 cells grown for a single propagation in LDS-medium was only slightly lower than that of cells grown in FCS-medium. Growth in LDS-medium induced a three-fold decrease in saturation density of SV3T3 and a twofold decrease in saturation density of SV3T3 Rev cells. A greater sensitivity of transformed versus untransformed cells to growth inhibition by lipid-depleted medium can also be observed in Rous sarcoma virus-transformed chick embryo fibroblasts (28) and in Kirsten sarcoma virus-transformed Balb/c3T3 cells (8) as compared to normal parental cells. On the other hand, Hatten, Horwitz, and Burger (6) found that proliferation was markedly inhibited in both Swiss 3T3 and SV101 3T3 cells grown in the presence of lipid-depleted serum. However, saturation densities of 3T3, SV3T3, and SV3T3 Rev cells grown in RS-medium were also reduced to levels similar to those of cells grown in LDS-medium. This result suggests that the inhibitory effect by LDS-medium is independent from the lipid depletion per se, and it is probably determined by an accumulation of toxic products or by the deterioration of growth factors due to the delipidation procedure. However, LDS- and RS-media might display their inhibitory effects on cell growth by distinct mechanisms. Whatever the mechanism underlying the growth inhibitory effect by LDS- or RS-media, the reason for the greater sensitivity of SV3T3 cells and SV3T3 Rev cells as compared to 3T3 cells to growth in manipulated media remains unclear.

Growth in LDS-medium did not change the cobblestone appearance of Balb/c3T3 cells and the flat morphology of SV3T3 Rev cells that are typical aspects of these cells grown in FCS-medium. Moreover, the SV3T3 cells maintained their characteristic spindle shape after being grown in LDS-medium, in contrast with the observation by Corwin, Humphrey, and Shloss (8) that growth in the presence of acetone-extracted serum changed the spindle morphology of Kirsten sarcoma virus-transformed Balb/c3T3 to a

flat and large appearance. Even growth in RS-medium did not change the typical morphology of the three cell lines.

Effect on lipid class content

In keeping with the result of a previous study (17), 3T3, SV3T3, and SV3T3 Rev cells grown in FCS-medium showed similar lipid class contents. The only exception was a lower phospholipid concentration in SV3T3 as compared with untransformed 3T3 cells (**Table 2**). After growth in LDS-medium, TG increased in 3T3 and SV3T3 cells but remained practically unmodified in SV3T3 Rev cells. FFA, on the other hand, increased in all cell lines. Moreover, 3T3, SV3T3, and SV3T3 Rev cells grown in LDS-medium maintained the same level of phospholipids but exhibited a lower concentration of free sterols as compared to cells grown in FCS-medium. In L cells grown for several subcultivations in the presence of delipidated serum, Sokoloff and Rothblat (29) also found an unchanged level of phospholipids and a reduction of sterols of the same order of magnitude as that observed in the cells analyzed in the present study. Persistence of an unchanged phospholipid concentration and maintenance of a certain level of sterols are probably crucial to sustain the proliferation in the absence of lipids from the growth medium.

The only sterol in free and esterified sterol fractions of 3T3, SV3T3, and SV3T3 Rev cells was cholesterol, regardless of whether the cells were grown in FCS- or LDS-media. From this point of view, the cellular system used in this study behaves like the diploid human embryonic fibroblasts line WI-38 and the SV40-transformed counterpart (30), while sterol synthesis did not proceed beyond the desmosterol stage in L cells grown in lipid-free media (31).

3T3, SV3T3, and SV3T3 Rev cells grown in RS-medium had phospholipid concentrations similar to those found in the cells grown in FCS-medium, but showed levels of free cholesterol comparable to those of cells grown in LDS-medium. Moreover, 3T3 cells and SV3T3 Rev cells grown in RS-medium increased their FFA and TG concentrations to a level similar to that found in cells grown in LDS-medium. In SV3T3 cells grown in RS-medium there was an increase of TG and FFA even greater than that observed when the cells were grown in LDS-medium. These findings with cells grown in RS-medium suggest that serum manipulation rather than lipid depletion accounts for the accumulation of TG and FFA and the reduction of free cholesterol in cells grown in LDS-medium, but the magnitude of TG and FFA changes in SV3T3 cells grown in RS-medium may indicate some specific effect by reconstituted serum.

TABLE 2. Lipid class content of Balb/c3T3, SV3T3, and SV3T3 Rev cells grown in media containing 10% complete fetal calf serum (FCS-serum), lipid-depleted fetal calf serum (LDS-medium), or reconstituted fetal calf serum (RS-medium)[a]

Lipid Class	FCS-Medium			LDS-Medium			RS-Medium		
	Balb/c3T3 (9)	SV3T3 (6)	SV3T3 Rev[b] (10)	Balb/c3T3 (7)	SV3T3 (5)	SV3T3 Rev[b] (7)	Balb/c3T3 (3)	SV3T3 (3)	SV3T3 Rev[b] (3)
Total phospholipids	166 ± 8.5	133 ± 6.6	148 ± 9.3	173 ± 23.5	133 ± 19.1	132 ± 16.0	199 ± 21.7	162 ± 34.9	162 ± 21.0
Free sterols[c]	23.8 ± 2.2	27.3 ± 3.4	27.2 ± 2.1	13.7 ± 3.3	18.3 ± 2.9	13.7 ± 1.4	15.7 ± 2.2	17.5 ± 2.3	15.6 ± 4.9
Esterified sterols[d]	1.3[e]	1.1[e]	1.7[e]	0.3[e]	1.4[e]	0.7[e]	NM[f]	NM[f]	NM[f]
Free fatty acids	5.5 ± 1.4	6.1 ± 1.6	5.1 ± 0.6	16.2 ± 2.9	19.4 ± 5.8	14.8 ± 3.0	21.9 ± 0.4	37.0 ± 7.3	15.9 ± 6.6
Triacylglycerols[g]	3.5 ± 0.5	4.4 ± 0.8	5.5 ± 0.7	14.5 ± 4.0	8.8 ± 1.9	8.0 ± 1.4	11.2 ± 2.1	31.5 ± 8.1	13.3 ± 2.8

[a] Concentrations, expressed as μg/mg protein, are the means ± SEM of the number of experiments listed in parentheses.
[b] As in Table 1.
[c] Determined colorimetrically. Regardless of the cell line and growth medium, free sterol fraction was found to be totally accounted for by cholesterol as observed after gas–liquid chromatography of the trimethylsilylether derivatives.
[d] Determined by quantitative gas–liquid chromatographic analysis using coprostanol as an internal standard. Cholesterol was the only sterol found in sterol esters of 3T3, SV3T3, and SV3T3 Rev cells grown in the presence of either complete or lipid-depleted serum.
[e] Value corresponds to only one experiment.
[f] NM, not measured.
[g] Triacylglycerols (TG) were evaluated from the quantitative gas–liquid chromatographic analysis of their fatty acid methyl esters and using the following formula: (*mol* TG *fatty acids*/3) × *molecular weight of* TG. *Molecular weight of* TG was calculated on the basis of the fatty acid composition.

As shown in **Table 3**, 3T3, SV3T3, and SV3T3 Rev cells grown in FCS-medium had similar phospholipid compositions, although SV3T3 and SV3T3 Rev cells had a higher percentage of PC, and SV3T3 Rev cells showed a lower proportion of DPG compared to 3T3 cells. The three cell lines grown in LDS-medium maintained approximately the same phospholipid pattern of the cells grown in FCS-medium, the only exception being an increase of DPG and SP in SV3T3 Rev cells. The phospholipid compositions of 3T3, SV3T3, and SV3T3 Rev cells were also unchanged after growth in RS-medium, with the only exception an increase of SP in SV3T3 Rev cells similar to that found when these cells were grown in LDS-medium.

Effect on phospholipid fatty acid composition

As noticed in a previous paper (17), the fatty acid profiles of PE and PC of SV3T3 and SV3T3 Rev cells grown in FCS-medium differ from those of untransformed 3T3 cells due to a higher proportion of 16:1 and 18:1 and to a lower level of 20:4. In PI + PS of SV3T3 cells compared to 3T3 cells there was a higher percentage of 18:1 and a lower level of 18:0.

When grown in LDS-medium, 3T3 cells showed an increase of 16:1 and 18:1 and a decrease of 20:4 and 22:5 + 6, accompanied by an accumulation of 20:3 (n-9) in all the phospholipid classes (**Table 4**), changes that are characteristics of EFA deficiency (32). It should be noted, however, that the accumulation of 20:3 (n-9) in 3T3 cells grown in LDS-medium did not compensate for the diminution of 20:4 as reported in tissues of EFA deficient animals (33–35).

In SV3T3 cells grown in LDS-medium there was an increase of 16:1 and 18:1 in PE and PI + PS. However, the magnitude of this change was lower than that found in 3T3 cells under the same growth conditions. In PC of SV3T3 cells grown in LDS-medium the increase of monoenoic acid was confined to 16:1. Moreover, SV3T3 cells grown in LDS-medium showed a decreased level of 20:4 in PE, PC, and PI + PS. The diminution of 20:4 in phospholipids after growth in LDS-medium was proportionally lower in SV3T3 than in 3T3 cells. Considering that in LDS-medium SV3T3 cells underwent a higher number of replications than 3T3 cells, it must be presumed that SV3T3 cells retained 20:4 more efficiently than 3T3 cells. It should be mentioned that L cells (36) and human fetal fibroblasts (37) have been reported to exhibit a much higher stability of their phospholipid acyl groups compared to fibroblasts of adult origin. Rosenthal and Somers (38), on the other hand, recently reported a similar degree of acyl group retention in normal and virally transformed cells. In contrast to the findings with 3T3 cells, in SV3T3 cells grown in LDS-medium 20:3 (n-9), although increased, remained a minor fatty acid in PE and PI + PS while it appeared unchanged in PC. The limited accumulation of 20:3 (n-9) in SV3T3 cells compared to 3T3 cells probably reflects a block of desaturation of 18:1, an observation in keeping with the finding that several transformed cell lines exhibit a deletion of Δ 6 desaturases (39, 40), a key enzyme in the synthesis of the different series of PUFA (41). Moreover, after growth in lipid-free media, HeLa cells (42), L cells (43), LM cells (44), and minimal deviation hepatoma 7288c cells (45) replaced 20:4 with only monoenoic acids.

SV3T3 Rev cells grown in LDS behaved like SV3T3 cells in that the increase of phospholipid monoenoic acids was less evident than in 3T3 cells grown in LDS-medium. Moreover, in PE, PC, and PI + PS of SV3T3 Rev cells grown in LDS-medium there was no accumulation of 20:3 (n-9), while the decrease of 20:4 was similar to that found in 3T3 cells.

The phospholipid fatty acid profiles of 3T3, SV3T3, and SV3T3 Rev cells grown in RS-medium were similar to those of cells grown in FCS-medium

TABLE 3. Phospholipid composition of Balb/c3T3, SV3T3, and SV3T3 Rev cells grown in media containing 10% complete fetal calf serum (FCS-medium), lipid-depleted fetal calf serum (LDS-medium), or reconstituted fetal calf serum (RS-medium)[a]

	FCS-Medium			LDS-Medium			RS-Medium		
Lipid Class	Balb/c3T3 (3)	SV3T3 (2)	SV3T3 Rev[b] (4)	Balb/c3T3 (3)	SV3T3 (2)	SV3T3 Rev[b] (4)	Balb/c3T3 (4)	SV3T3 (4)	SV3T3 Rev[b] (4)
DPG	3.3 ± 0.5	2.2 ± 0.3	1.1 ± 0.1	5.4 ± 2.8	2.6 ± 0.1	2.9 ± 0.4	3.9 ± 0.5	5.1 ± 1.0	3.9 ± 1.1
PE	24.0 ± 0.7	19.7 ± 3.0	21.8 ± 1.1	16.8 ± 2.5	18.8 ± 5.4	18.8 ± 3.0	20.9 ± 0.7	19.6 ± 1.2	26.6 ± 2.9
PC	44.2 ± 2.2	54.6 ± 2.0	53.4 ± 1.1	50.2 ± 5.5	53.9 ± 0.7	45.2 ± 3.4	49.0 ± 3.7	47.8 ± 3.5	44.9 ± 3.4
PI + PS	13.5 ± 1.0	11.7 ± 4.3	14.0 ± 1.7	17.4 ± 2.1	11.8 ± 2.8	12.0 ± 1.7	16.1 ± 1.8	18.1 ± 1.8	11.5 ± 1.6
SP	14.0 ± 3.5	11.8 ± 0.5	7.6 ± 0.6	10.1 ± 2.3	11.2 ± 7.0	16.2 ± 2.7	10.1 ± 2.7	9.4 ± 2.0	13.1 ± 2.1
LPC	1.0 ± 1.0	ND[c]	2.1 ± 0.7	ND[c]	1.9 ± 0.4	4.9 ± 3.5	ND[c]	ND[c]	ND[c]

[a] Values, expressed as mole percentage, are the means ± SEM of a number of experiments listed in parentheses. Data of individual phospholipid classes were calculated from the molar content of their fatty acids as determined by quantitative gas–liquid chromatography, taking into account the different fatty acid mole contribution in the various phospholipid classes.

[b] As in Table 1.

[c] ND, not detectable under the analytical conditions used.

TABLE 4. Fatty acid composition of phosphatidylethanolamine (PE), phosphatidylcholine (PC), and phosphatidylinositol + phosphatidylserine (PI + PS) from Balb/c3T3, SV3T3, and SV3T3 Rev cells grown in media containing 10% complete fetal calf serum (FCS-medium), lipid-depleted fetal calf serum (LDS-medium), or reconstituted fetal calf serum (RS-medium)[a]

Cell Line	Phospholipid Class	Fatty Acids[b] 16:0	16:1	18:0	18:1	18:2	20:2	20:3 (n-9)	20:3 (n-6)	20:4	22:5 + 6	Others[c]
						FCS-medium						
Balb/c3T3	PE (7)	7.6 ± 1.0	2.1 ± 0.4	20.5 ± 1.5	15.4 ± 2.2	0.9 ± 0.1	0.2 ± 0.2	4.0 ± 1.0	0.5 ± 0.2	22.6 ± 2.5	14.8 ± 1.0	11.4
	PC (7)	32.0 ± 1.5	9.3 ± 0.7	11.5 ± 0.5	25.9 ± 2.1	1.6 ± 0.3	0.1 ± 0.1	1.2 ± 0.3	0.5 ± 0.1	8.0 ± 1.6	4.6 ± 0.8	5.3
	PI + PS (7)	4.3 ± 0.6	2.8 ± 0.8	46.6 ± 2.9	15.5 ± 0.9	1.7 ± 0.7	0.3 ± 0.1	3.4 ± 1.0	1.1 ± 0.2	15.5 ± 2.9	5.9 ± 0.7	2.9
SV3T3	PE (7)	12.8 ± 1.7	5.1 ± 0.7	16.2 ± 0.9	29.5 ± 1.6	1.8 ± 0.3	0.3 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	11.2 ± 1.0	11.7 ± 0.8	9.2
	PC (7)	32.0 ± 0.9	12.0 ± 0.9	8.4 ± 0.6	33.3 ± 1.6	1.9 ± 0.2	0.1 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	3.8 ± 0.5	2.0 ± 0.2	5.7
	PI + PS (7)	8.3 ± 1.2	2.5 ± 0.4	38.9 ± 1.2	22.1 ± 2.1	1.3 ± 0.3	0.2 ± 0.1	2.6 ± 0.6	1.1 ± 0.3	12.4 ± 0.2	7.4 ± 0.6	3.1
SV3T3 Rev[d]	PE (7)	13.6 ± 1.9	5.6 ± 0.8	18.6 ± 1.5	31.4 ± 2.0	1.0 ± 0.3	0.7 ± 0.2	3.9 ± 0.9	0.1 ± 0.1	10.0 ± 1.2	7.7 ± 0.7	7.4
	PC (7)	32.3 ± 2.3	13.6 ± 1.0	8.2 ± 0.6	36.3 ± 1.7	1.2 ± 0.2	0.4 ± 0.1	1.0 ± 0.3	0.1 ± 0.0	2.0 ± 0.4	1.7 ± 0.3	3.2
	PI + PS (7)	4.9 ± 0.5	3.0 ± 0.3	44.6 ± 4.0	26.0 ± 2.3	0.3 ± 0.1	0.6 ± 0.3	6.3 ± 1.3	0.5 ± 0.1	7.6 ± 0.9	4.9 ± 0.6	1.3
						LDS-medium						
Balb/c3T3	PE (7)	10.6 ± 0.9	7.4 ± 0.5	16.0 ± 1.0	30.4 ± 2.5	0.7 ± 0.3	0.9 ± 0.3	9.8 ± 1.6	0.2 ± 0.2	9.6 ± 2.0	4.2 ± 0.7	10.2
	PC (8)	29.2 ± 1.7	16.6 ± 1.2	9.0 ± 0.7	31.5 ± 1.7	1.3 ± 0.2	0.4 ± 0.2	2.8 ± 0.8	0.1 ± 0.0	3.1 ± 0.8	1.1 ± 0.3	4.9
	PI + PS (8)	11.3 ± 2.2	5.4 ± 0.5	33.1 ± 1.5	28.1 ± 2.1	0.8 ± 0.6	0.6 ± 0.3	8.6 ± 1.6	0.8 ± 0.3	5.8 ± 1.0	2.5 ± 0.3	3.0
SV3T3	PE (7)	15.3 ± 1.5	11.1 ± 1.7	14.4 ± 0.8	34.1 ± 0.5	1.6 ± 0.6	3.2 ± 2.2	2.4 ± 0.7	0.8 ± 0.2	6.7 ± 0.9	2.4 ± 0.6	8.0
	PC (7)	31.9 ± 0.9	18.4 ± 3.0	6.4 ± 0.6	32.6 ± 2.7	0.9 ± 0.2	0.9 ± 0.5	0.6 ± 0.2	0.1 ± 0.0	2.4 ± 0.7	1.4 ± 0.4	4.4
	PI + PS (7)	12.6 ± 1.7	5.4 ± 1.0	28.7 ± 1.9	30.7 ± 2.6	1.2 ± 0.2	3.4 ± 2.2	4.5 ± 1.5	0.8 ± 0.3	6.1 ± 2.2	2.9 ± 0.4	4.7
SV3T3 Rev[d]	PE (8)	15.2 ± 2.2	9.8 ± 1.1	15.3 ± 0.7	40.6 ± 3.4	0.6 ± 0.2	1.2 ± 0.6	4.0 ± 1.0	0.5 ± 0.2	4.0 ± 0.7	1.3 ± 0.3	7.2
	PC (9)	36.3 ± 2.9	17.7 ± 1.9	7.8 ± 1.4	32.1 ± 3.4	0.5 ± 0.2	0.8 ± 0.5	0.6 ± 0.3	0.1 ± 0.0	0.4 ± 0.2	0.2 ± 0.1	3.5
	PI + PS (7)	14.0 ± 2.7	6.2 ± 0.6	33.9 ± 3.4	28.9 ± 4.8		3.7 ± 2.4	4.7 ± 1.4	1.5 ± 0.4	2.1 ± 0.7	0.7 ± 0.3	4.9

[a] Values, expressed as percentages by weight of fatty acid methyl esters, are the means ± SEM of a number of experiments listed in parentheses.
[b] Fatty acids are indicated by number of carbon atoms:number of double bonds.
[c] Others include 14:0, 20:5, 22:3, 22:4, and C16:0 and C18:0 aldehydogenic chains.
[d] See note *b* in Table 1.

(figures not reported in Table) indicating that no manipulation of the serum other than lipid depletion accounts for the changes in fatty acid composition found in cells grown in LDS-medium.

Table 4 also shows that the level of 18:2 in 3T3, SV3T3, and SV3T3 Rev cells was almost the same regardless of whether the cell lines were grown in FCS- or in LDS-media. Since isomeric composition of 18:2 was not analyzed in this study, it is impossible to say whether the level of 18:2 in cells grown in LDS-medium is accounted for by the accumulation of the desaturation product of 18:1 (n-9) or by the persistence of residual 18:2 (n-6), both conditions being observed in L cells grown in media supplemented with lipid-free serum (46, 47).

In conclusion, the following considerations can be drawn from the results of the present investigation: *1*) Balb/c3T3 cells, an established cell line which exhibits density dependent growth inhibition, behave like mammalian cells in vivo in that they show, when grown in lipid-free media, changes in the phospholipid fatty acid profiles similar to those found in tissues of animals kept on EFA-deficient diet. In particular, accumulation of 20:3 (n-9) in Balb/c3T3 cells grown in LDS-medium indicates that these cells are able to synthesize PUFA from oleic acid, in contrast to the generalization that propagation in tissue culture induces a loss of desaturating enzymes involved in the biosynthesis of the different series of PUFA (48); *2*) The slight accumulation of 20:3 (n-9) in SV3T3 cells grown in LDS-medium, also observed in other transformed cell lines (42–45), suggests that a block of the biosynthesis of higher PUFA is associated with transformation, although there are a few examples of transformed cell lines which synthesize 20:4 from 18:2 (40) and accumulate 20:3 (n-9) after growth in lipid-free media (49). Furthermore, SV3T3 cells grown in LDS-medium maintained the transformation-associated change in the monoenoic/20 and 22 polyunsaturated acid ratio already observed in SV3T3 cells grown in FCS-medium (17); *3*) The lack of 20:3 (n-9) accumulation in SV3T3 Rev cells grown in LDS-medium suggests that the integration of SV40 genoma in Balb/c3T3 cells was sufficient to induce a block in the biosynthesis of higher PUFA whether or not biological characteristics of transformation were expressed.

The authors wish to thank Prof. A. Fonnesu, Chairman of the Institute of General Pathology, University of Florence, for his interest in this work. This work was supported by grant No. 78.0286396 of Finalized Project "Control of Tumor Growth" sponsored by Consiglio Nazionale delle Ricerche.

Manuscript received 6 March 1980 and in revised form 20 November 1980.

REFERENCES

1. Howard, B. V., and W. J. Howard. 1974. Lipid metabolism in cultured cells. *Adv. Lipid Res.* **12:** 51–96.
2. Howard, B. V., and W. J. Howard. 1975. Lipids in normal and tumor cells in culture. *Prog. Biochem. Pharmacol.* **10:** 135–166.
3. Bailey, J. M. 1977. Cultured cells. *In* Lipid Metabolism in Mammals. F. Snyder, editor. Plenum Press, New York and London. **2:** 323–352.
4. Holley, R. W., J. H. Baldwin, and J. Kiernan. 1974. Control of growth of a tumor cell by linoleic acid. *Proc. Natl. Acad. Sci. USA.* **71:** 3976–3978.
5. Horwitz, A. F., M. E. Hatten, and M. M. Burger. 1974. Membrane fatty acid replacements and their effect on growth and lectin-induced agglutinability. *Proc. Natl. Acad. Sci. USA* **71:** 3115–3119.
6. Hatten, M. E., A. F. Horwitz, and M. M. Burger. 1977. The influence of membrane lipids on the proliferation of transformed and untransformed cell lines. *Exp. Cell Res.* **107:** 31–34.
7. Cornell, R., G. L. Grove, G. H. Rothblat, and A. F. Horwitz. 1977. Lipid requirement for the cell cycling. The effect of selective inhibition of lipid synthesis. *Exp. Cell Res.* **109:** 299–307.
8. Corwin, L. M., L. P. Humphrey, and J. Shloss. 1977. Effect of lipids on the expression of cell transformation. *Exp. Cell Res.* **108:** 341–347.
9. Williams, R. E., G. H. Rittenhouse, K. K. Iwata, and C. F. Fox. 1977. Effects of low temperature and lipid modification on the proliferation of cultured mammalian cells. *Exp. Cell Res.* **107:** 95–104.
10. Doi, O., F. Doi, F. Schroeder, A. W. Alberts, and R. P. Vagelos. 1978. Manipulation of fatty acid composition of membrane phospholipid and its effects on the cell growth in mouse LM cells. *Biochim. Biophys. Acta.* **509:** 239–250.
11. Horwitz, A. F. 1977. The structural and functional roles of lipids in the surfaces of animal cells grown in vitro. *In* Growth, Nutrition and Metabolism of Cells in Culture. G. H. Rothblat and V. J. Cristofalo, editors. Academic Press, New York. 3:109–148.
12. Horwitz, A. F., A. Wight, P. Ludwig, and R. Cornell. 1978. Interrelated lipid alterations and their influence on the proliferation and fusion of cultured myogenic cells. *J. Cell Biol.* **77:** 334–357.
13. Curtis, A. S. G., C. Chandler, and N. Picton. 1975. Cell surface lipids and adhesion. III. The effects on the cell adhesion of changes in plasmalemmal lipids. *J. Cell Sci.* **18:** 375–384.
14. Hoover, R. L., R. Lynch, and M. J. Karnovsky. 1977. Decrease in adhesion of cells cultured in polyunsaturated fatty acids. *Cell.* **12:** 295–300.
15. Li, J. K. K., R. E. Williams, and C. F. Fox. 1975. Effects of temperature and host lipid composition on the infection of cells by Newcastle disease virus. *Biochem. Biophys. Res. Commun.* **62:** 470–477.
16. Tombaccini, D., S. Ruggieri, A. Fallani, and G. Mugnai. 1980. Concanavalin A-mediated agglutinability of Balb/c3T3 cells grown in media supplemented with different phosphatidylcholines. *Biochim. Biophys. Res. Commun.* **96:** 1109–1115.
17. Ruggieri, S., R. Roblin, and P. H. Black. 1979. Lipids of whole cells and plasma membrane fractions from Balb/

c3T3, SV3T3 and Con A-selected revertant cells. *J. Lipid Res.* **20:** 772–783.
18. Culp, L. A., and P. H. Black. 1972. Contact-inhibited revertant cell lines isolated from Simian virus 40-transformed cells. III. Concanavalin A-selected revertant cells. *J. Virol.* **9:** 611–620.
19. Schultz, A. R., and L. A. Culp. 1973. Contact-inhibited revertant cell lines isolated from SV40-transformed cells. V. Contact inhibition of sugar transport. *Exp. Cell Res.* **81:** 95–103.
20. Roblin, R., S. O. Albert, N. A. Gelb, and P. H. Black. 1975. Cell surface changes correlated with density-dependent growth inhibition. Glycosoaminoglycan metabolism in 3T3, SV3T3, and Con A-selected revertant cells. *Biochemistry.* **14:** 347–357.
21. Folch, J., M. Lees, and G. M. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
22. Beroza, M., and B. A. Bierl. 1967. Rapid determination of olefin position in organic compounds in microgram range by ozonolysis and gas chromatography. *Anal. Chem.* **39:** 1131–1135.
23. Wood, R., J. Falch, and R. Wiegand. 1974. Lipids of cultured hepatoma cells. V. Distribution of isomeric monoene fatty acids in individual lipid classes. *Lipids* **9:** 987–992.
24. Privett, O. S., M. L. Blank, and O. Romanus. 1963. Isolation analysis of tissue fatty acids by ultramicro-ozonolysis in conjunction with thin-layer chromatography and gas–liquid chromatography. *J. Lipid Res.* **4:** 260–265.
25. Cramer, K., and B. Isaksson. 1959. An evaluation of the Theorell method for the determination of total serum cholesterol. *Scand. J. Clin. Lab. Invest.* **11:** 213–216.
26. Martin, G. B., and B. M. Doty. 1949. Determination of inorganic phosphate. Modification of isobutyl alcohol procedure. *Anal. Chem.* **21:** 965–967.
27. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
28. Hale, A. H., J. E. Pessin, F. Palmer, M. J. Weber, and M. Glaser. 1977. Modification of the lipid composition of normal and Rous sarcoma virus-infected cells. Effects on transformation-associated membrane properties. *J. Biol. Chem.* **252:** 6190–6200.
29. Sokoloff, L., and G. H. Rothblat. 1974. Sterol to phospholipid molar ratios of L cells with qualitative and quantitative variations of cellular sterol. *Proc. Soc. Exp. Biol. Med.* **146:** 1166–1172.
30. Rothblat, G. H., R. Boyd, and C. Deal. 1971. Cholesterol biosynthesis in WI-38 and WI-38VA13A tissue culture cells. *Exp. Cell Res.* **67:** 436–440.
31. Rothblat, G. H., C. H. Burns, R. L. Conner, and J. R. Landrey. 1970. Desmosterol as the major sterol in L-cell mouse fibroblasts grown in sterol-free culture medium. *Science.* **169:** 880–881.
32. Holman, R. T. 1967. Essential fatty acid deficiency. *In* Progress in the Chemistry of Fats and Other Lipids. R. T. Holman, editor. Pergamon Press, Oxford, London. **9:** 279–348.
33. Biran, L. A., W. Bartley, C. W. Carter, and A. Renshaw. 1964. Studies on essential fatty acid deficiency. Effect of the deficiency in various rat tissues and the influence of dietary supplementation with essential fatty acids on deficient rats. *Biochem. J.* **93:** 492–498.
34. Johnson, R. M., and T. Ito. 1965. Effects of a nutritional deficiency of unsaturated fats on the distribution of fatty acids in rat liver mitochondrial phospholipids. *J. Lipid Res.* **6:** 75–79.
35. Ruggieri, S., A. Fallani, and D. Tombaccini. 1976. Effect of essential fatty acid deficiency on the lipid composition of the Yoshida ascites hepatoma (AH130) and of the liver and blood plasma from host and normal rats. *J. Lipid Res.* **17:** 456–466.
36. Lynch, R. D., E. E. Schneeberger, and R. P. Geyer. 1976. Complete retention of phospholipid acyl groups by mammalian cells in culture. *Biochemistry.* **15:** 193–200.
37. Rosenthal, M. D., and R. P. Geyer. 1976. Phospholipid acyl group stability in cultured fibroblasts. Differences between human cell lines of fetal and adult origin. *Biochim. Biophys. Acta.* **441:** 465–476.
38. Rosenthal, M. D., and K. D. Somers. 1979. Retention of phospholipid acyl groups is not characteristic of neoplastic cells in culture. *Biochim. Biophys. Acta* **574:** 356–360.
39. Dunbar, L. M., and J. M. Bailey. 1975. Enzyme deletions and essential fatty acid metabolism in cultured cells. *J. Biol. Chem.* **250:** 1152–1153.
40. Maeda, M., O. Doi, and Y. Akamatzu. 1978. Metabolic conversion of polyunsaturated fatty acids in mammalian cultured cells. *Biochim. Biophys. Acta.* **530:** 153–164.
41. Brenner, R. R. 1974. The oxidative desaturation of unsaturated fatty acids in animals. *Mol. Cell. Biochem.* **3:** 41–52.
42. Kagawa, Y., T. Takaoka, and H. Katsuta. 1970. Absence of essential fatty acids in mammalian cell strains cultured in lipid- and protein-free chemically defined synthetic media. *J. Biochem.* **68:** 133–136.
43. Lengle, E., and R. P. Geyer. 1972. Comparison of cellular lipids of serum-free strain L mouse fibroblasts. *Biochim. Biophys. Acta.* **260:** 608–616.
44. Anderson, R. E., R. B. Cumming, M. Walton, and F. Snyder. 1969. Lipid metabolism in cells grown in tissue culture: *O*-alkyl, *O*-alk-1-enyl, and acyl moieties of LM cells. *Biochim. Biophys. Acta.* **176:** 491–501.
45. Wood, R., and J. Falch. 1973. Lipids of cultured hepatoma cells. II. Effect of media lipids on cellular phospholipids. *Lipids.* **8:** 702–710.
46. Bailey, J. M., and J. Menter. 1967. Lipid metabolism in cultured cells. VII. Linoleic acid content of cells grown on lipid-free synthetic medium. *Proc. Soc. Exp. Biol. Med.* **125:** 101–105.
47. Tsai, P., and R. P. Geyer. 1977. Fatty acid synthesis and metabolism of phospholipid acyl groups in strain L mouse fibroblasts. *Biochim. Biophys. Acta.* **489:** 381–389.
48. Harary, I., L. E. Gerschenson, D. F. Haggerty, Jr., W. Desmond, and J. F. Mead. 1967. Fatty acid metabolism and function in cultured heart and HeLa cells. *In* Lipid Metabolism in Tissue Culture Cells. G. H. Rothblat and D. Kritchevsky, editors. The Wistar Institute Press, Philadelphia. **6:** 17–31.
49. Bailey, J. M., B. V. Howard, L. M. Dunbar, and S. F. Tillman. 1972. Control of lipid metabolism in cultured cells. *Lipids.* **7:** 125–134.