IN VITRO Vol. 20, No. 9, September 1984

CHANGES IN SERUM INFLUENCE THE FATTY ACID COMPOSITION OF ESTABLISHED CELL LINES

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(Received 12 May 1984; accepted 4 June 1984)

SUMMARY

The fatty acid composition of different kinds of commercially available serum used to supplement cell culture media differs widely. As compared with fetal bovine serum, horse and bovine calf serum have a very high content of linoleic acid (18:2) and are low in arachidonic acid (20:4). (Fatty acids are abbreviated as number of carbon atoms:number of double bonds). Swine serum contains substantial amounts of both 18:2 and 20:4. Only fetal bovine serum contains more than 1% docosahexaenoic acid (22:6). Considerable differences in fatty acid composition occur when cells are grown in media containing any of these different serum supplements. The 18:2 and 20:4 content of 3T3 mouse fibroblast phospholipids is highest when the medium contains horse serum, intermediate with bovine calf serum, and lowest with swine or fetal bovine serum. Likewise, the highest phospholipid 18:2 content in Madin-Darby canine kidney cells (MDCK) occurs when the medium contains horse serum. With MDCK cells, however, growth in swine serum produces the highest 20:4 content. The 3T3 cell phospholipids accumulate more than 1% 22:6 only when the medium contains fetal bovine serum, whereas in no case do the MDCK cell phospholipids accumulate appreciable amounts of 22:6. The fact that the cellular fatty acid composition is likely to change should be taken into account when changes are contemplated in the serum used to grow established cell lines.

Key words: 3T3 cells; MDCK cells; fetal bovine serum; horse serum; swine serum; calf serum.

INTRODUCTION

Appreciable changes in the fatty acid composition of animal cells can be produced by adding specific fatty acids in unesterified form to the culture medium (9,31,35). The fatty acid composition of the membrane phospholipids is modified by this procedure, and this is associated with changes in membrane fluidity (4,18,28,36). These types of fatty acid compositional modifications have been correlated with effects on cell growth (7), fusion (14), phagocytosis (21,27), mitogenic stimulation (20), amino acid transport (16,17), endocytosis (23), prostaglandin production (6,29), infectivity of enveloped viruses (19), tumor cell resistance to complement-mediated antibody destruction (26), opioid binding (13), insulin receptor number and affinity (12), and complement-mediated cytolysis (24).

A number of studies have shown that cultured cells derive most of their lipids from the serum present in the growth medium (2,22,32). The sera obtained from different animal species have considerably different fatty acid compositions (32). It is not known, however, whether extensive modifications in fatty acid composition, such as those produced by unesterified fatty acid supplements, will occur if cells are grown in culture media supplemented with different kinds of serum. Most of the fatty acid in serum is present as components of lipid esters, which are contained in lipoproteins, and only a small amount is in the form of unesterified fatty acid. Therefore, it is possible that the different sera might not

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produce substantial changes in cellular fatty acid composition. Information regarding this point is important because it may become necessary to change the type of serum used to supplement culture media if the supply of fetal bovine serum is severely curtailed. In the present study, we have examined the variability in fatty acid composition of fetal bovine, bovine calf, horse, and swine serum. In addition, we have determined the extent to which phospholipid fatty acid composition varies when cells are cultured in media supplemented with each of these types of serum.

MATERIALS AND METHODS

Cells. Madin-Darby canine kidney tubular epithelial (MDCK) cells (ATCC CCL 34) and 3T3 mouse fibroblasts (ATCC CCL 92) were obtained from the American Type Culture Collection (Rockville, MD). For each cell line used, the species of origin and absence of contamination by a second cell line were verified by isoenzyme electrophoresis using the Corning Authentikit (Corning Medical and Scientific, East Walpole, MA). All cell lines were screened at regular intervals for mycoplasma contamination by the method of Velleca et al. (34) and were found to be negative in every instance.

Stock cultures of MDCK cells were maintained in minimum essential medium with Earle's salts (GIBCO, Grand Island, NY); 3T3 cell stocks were maintained in Dulbecco's minimum essential medium (GIBCO). In each case this basic growth medium was supplemented by 10% serum (fetal bovine unless otherwise specified), MEM nonessential amino acids (GIBCO), MEM vitamin solution (GIBCO), 15 m*M* 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) (Sigma, St. Louis, MO), 2 m*M* glutamine, and 50 μg/ml garamycin (Schering Corp., Kenilworth, NJ). Stock cultures were maintained at 37° C in a humidified atmosphere containing 5% CO_2. The MDCK cell stocks were routinely subcultured weekly and 3T3 cell twice weekly by trypsinization and reseeding at a 1:10 dilution. Stock cultures of 3T3 cells were never allowed to become confluent. For experiments, each cell line was grown for four passages in medium containing the serum that was to be studied before experimental cultures were seeded. After seeding, all experimental cultures were grown for 1 wk (including one refeeding) before cells were harvested.

Phospholipid composition. Confluent cell monolayers were washed three times with cold phosphate buffered saline (PBS), then harvested by scraping with a rubber policeman and pelleted by centrifugation. The cell pellet was resuspended in 1 ml of buffer, and lipids were extracted with chloroform:methanol, 2:1 vol/vol (11). Phospholipids and neutral lipids were separated by thin layer chromatography on silica gel G plates (Alltech Associates, Deerfield, IL) using a solvent system of mixed hexanes:diethyl ether:acetic acid:methanol, 170:40:4:4, vol/vol (31). The phospholipid band was eluted from the gel with chloroform:methanol, 1:1 (vol/vol), and then saponified and methylated (31). Fatty acid methyl esters were separated by gas liquid chromatography using a Hewlett Packard model 5700 gas liquid chromatograph containing a 1.8 m glass column (2 mm i.d.) packed with 10% SP-2330 on 100/120 mesh Chromasorb W-AW (Supelco, Bellefonte, PA). Peak assignments were made by comparing retention times with those of fatty acid standards (NuChek Prep, Elysian, MN). Peak areas were determined with a Hewlett Packard Model 3380-S integrator-recorder and are reported as weight percentages.

Chloroform-methanol extracts of cells were separated into individual phospholipid classes by the method of Fine and Sprecher (10), using boric acid-impregnated silica gel H plates developed in a solvent mixture of chloroform:methanol:water:ammonium hydroxide, 120:75:6:2 (vol/vol). Lipid phosphorus in each fraction was measured by the method of Chalvardjian and Rudnicki (5).

Serum. Samples of a large number of lots of HyClone fetal bovine serum, as well as horse, swine, and bovine calf serum, were provided by Sterile Systems (Logan, UT). After heat inactivation of serum at 56° C for 30 min, samples from each lot of serum were extracted with chloroform:methanol, 2:1, vol/vol, as described above. An aliquot from each serum lipid extract was taken for fatty acid analysis. The fatty acids were saponified and methylated and the fatty acyl composition determined by gas liquid chromatography (31).

RESULTS

Serum fatty acids. The fatty acid compositions of fetal bovine, bovine calf, horse, and swine serum are shown in Table 1. Considerable differences are apparent. Fetal bovine serum has consistently higher percentages of arachidonic (20:4) and oleic (18:1) acids than either bovine

TABLE 1

FATTY ACID COMPOSITION OF DIFFERENT SERA

	Composition (%)[a]						
	Fetal Bovine (49)[b]		Bovine Calf (16)		Horse (11)		Swine (2)
Fatty Acid	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Average
Individual acids[c]							
16:0	20.1 ± 0.35	15.6–24.6	13.2 ± 0.32	9.6–14.7	15.8 ± 0.90	11.4–21.2	15.5
16:1	6.1 ± 0.10	3.9–7.5	1.9 ± 0.07	1.1–2.3	2.6 ± 0.25	1.4–4.2	2.3
18:0	12.6 ± 0.15	11.2–14.8	13.7 ± 0.19	12.9–15.6	16.2 ± 0.42	13.8–17.8	14.1
18:1	28.1 ± 0.17	25.9–31.0	16.2 ± 0.27	14.2–18.0	16.9 ± 0.95	12.2–22.7	28.7
18:2	6.2 ± 0.09	4.9–8.1	44.2 ± 0.49	41.2–47.7	39.1 ± 1.56	31.3–47.7	20.9
18:3	0.3 ± 0.03	0.1–0.7	1.0 ± 0.05	0.8–1.3	4.8 ± 0.40	2.8–6.8	0.4
20:4	10.4 ± 0.16	8.3–12.5	3.7 ± 0.10	3.2–4.3	1.1 ± 0.05	0.7–1.3	11.5
22.6	4.1 ± 0.11	2.6–6.1	0.5 ± 0.01	0.4–0.6	<0.1	0–0.1	0.6
Classes							
Saturated	34.0	30.4–37.3	28.1	25.3–30.6	32.8	29.7–36.8	31.1
Monounsaturated	34.3	31.7–36.9	18.6	16.7–20.6	19.6	15.6–26.2	31.3
Polyunsaturated	27.8	26.2–33.6	52.0	48.1–55.9	45.6	38.4–51.1	36.2
Unsaturation							
Index[d]	1.47	1.27–1.71	1.39	1.33–1.48	1.22	1.10–1.30	1.37
Mean chain length[e]	18.0	17.9–18.2	17.8	17.6–18.0	17.6	17.4–17.8	18.0

[a] The percentage values for the individual fatty acids contained in each type of serum do not add up to 100% because only the most prevalent fatty acids are listed.

[b] Number of lots of serum tested.

[c] The fatty acids are abbreviated as number of carbon atoms:number of double bonds.

[d] Average number of double bonds in each fatty acid.

[e] Average number of carbon atoms in each fatty acid.

calf or horse serum. (Fatty acids are abbreviated as number of carbon atoms:number of double bonds). By contrast, bovine calf and horse serum consistently have the highest percentages of linoleic acid (18:2). Swine serum contains about the same percentage of 18:1 and 20:4 as fetal bovine serum but, in addition, has a moderately high percentage of 18:2. Only fetal bovine serum has appreciable amounts of docosahexaenoic acid (22:6). Although the SEM values for the fatty acid composition of each type of serum are small, considerable variations from the mean occurred in an occasional sample. This is apparent in the wide range of values for 20:4 and 22:6 in fetal bovine serum, 18:1 in horse serum, and palmitic acid (16:0) and 18:2 in fetal bovine, horse, and bovine calf serum.

Cell phospholipid fatty acyl composition. Considerable changes in cell phospholipid fatty acyl composition were observed when cultures were grown with different serum supplements. Results obtained for 3T3 mouse fibroblasts and MDCK cells are shown in Table 2. The 3T3 cell phospholipids exhibited a wide variation in 18:2 percentage composition, being highest when the cultures were grown with horse serum, intermediate with bovine calf serum, and lowest with either fetal bovine or swine serum. A similar distribution was noted for 20:4. Only 3T3 cells grown with fetal bovine serum accumulated appreciable amounts of 22:6. The phospholipids of 3T3 cells grown with horse serum contained the highest percentages of total polyunsaturates, the highest unsaturation index, and the highest fatty acid mean chain length.

The MDCK cells exhibited generally similar changes in phospholipid fatty acyl composition to those observed in 3T3 cells, with a few potentially important exceptions. For example, the percentage of 18:2 was highest when the MDCK cells were grown in swine serum, not horse serum. The unsaturation index also was highest when the cells were grown in swine serum even though the total polyunsaturated fatty acid content was highest with horse serum.

The content of individual phospholipid classes of 3T3 and MDCK cells is presented in Table 3. The total lipid phosphorus content and its distribution among the different phospholipid classes is very similar in the two cell lines. Therefore, differences in phospholipid content or composition cannot account for the differences seen in polyunsaturated fatty acid content between the two lines.

Cell growth and properties. With both 3T3 and MDCK cells, the highest growth rates occurred in the presence of swine serum. Dome formation in MDCK cultures also was greatest in swine serum. Growth of 3T3 and MDCK cells was more rapid in horse serum as compared with fetal bovine serum. The growth rate of 3T3 cells was very slow in bovine calf serum, and MDCK cells failed to grow in bovine calf serum.

DISCUSSION

We have reported previously that the single lots of fetal bovine, bovine calf, horse, and swine serum used by our laboratory to prepare culture media have sizable differences in fatty acid composition (32). The present findings indicate that although some variation can occur among different lots of a given serum, the trends reported previously are generally typical for serum of that species. As compared with fetal bovine serum, horse and bovine calf serum are quite low in 20:4, the substrate for synthesis of the main classes of prostaglandins and leukotrienes. By contrast, they are very high in 18:2, the essential polyunsaturated fatty acid that can be used by some but not all cultured cells for the synthesis of 20:4 (8,15,24,25,30). Swine serum, like fetal bovine serum, has a relatively high 20:4 content, but it also has an intermediate 18:2 content. Only fetal bovine serum has an appreciable amount of 22:6, the main *n*-3 polyunsaturate contained in retinal and neural cells (1,33).

Replacement of fetal bovine serum in the growth medium of cultured 3T3 and MDCK cells by horse, bovine calf, or swine serum produced appreciable changes in the phospholipid fatty acyl composition. Although the resulting cellular phospholipid fatty acyl compositions reflect the fatty acid composition of the added serum, they do not directly mimic the serum fatty acid composition. This suggests that there is either selectivity in the uptake of different fatty acids contained in the serum or selectivity in their incorporation after uptake. Furthermore, the composition determined in the 3T3 cells was somewhat different from that observed in MDCK cells, indicating that the precise modifications that will occur cannot always be predicted from information obtained with a single cell line.

The phospholipid fatty acyl modifications produced by changes in the type of serum added to the medium are almost as extensive as those observed when the medium is supplemented

TABLE 2

PHOSPHOLIPID FATTY ACYL COMPOSITION OF CELL GROWN IN CULTURE MEDIA CONTAINING DIFFERENT SERUM SUPPLEMENTS

	Composition (%)[a]						
	3T3				MDCK		
Fatty Acid	Fetal Bovine[b]	Bovine Calf	Horse	Swine	Fetal Bovine	Horse	Swine
Individual acids[c]							
16:0	17.9 ± 0.55	17.6 ± 0.18	12.6 ± 0.41	17.9 ± 0.47	13.8 ± 0.17	14.1 ± 1.05	13.3 ± 0.23
16:1	5.2 ± 0.22	2.4 ± 0.05	2.3 ± 0.15	4.9 ± 0.06	4.1 ± 0.22	1.8 ± 0.20	3.7 ± 0.65
18:0	15.5 ± 0.44	19.9 ± 0.35	23.8 ± 0.68	14.6 ± 0.30	16.3 ± 0.18	19.5 ± 0.46	16.6 ± 0.39
18:1	34.9 ± 1.50	27.9 ± 0.28	21.1 ± 1.00	34.3 ± 0.24	45.1 ± 0.50	26.9 ± 0.57	30.0 ± 0.23
18:2	2.6 ± 0.20	8.5 ± 0.11	12.3 ± 0.97	3.3 ± 0.35	2.0 ± 0.08	16.2 ± 0.87	5.0 ± 0.39
18:3	<0.1	<0.1	1.3 ± 0.21	0.1 ± 0.03	0.2 ± 0.05	0.8 ± 0.03	0.2 ± 0.03
20:4	7.6 ± 0.63	14.0 ± 0.59	17.4 ± 0.39	9.8 ± 0.22	4.2 ± 0.37	9.0 ± 0.47	12.8 ± 0.26
22:6	3.0 ± 0.37	0.5 ± 0.05	<0.1	0.4 ± 0.06	1.0 ± 0.14	0.1 ± 0	0.9 ± 0.19
Classes							
Saturated	34.2	38.1	37.2	33.4	31.1	34.0	31.1
Monounsaturated	41.2	31.2	23.8	40.6	51.1	29.8	34.9
Polyunsaturated	21.7	29.3	38.4	23.5	15.7	34.3	29.0
Unsaturation Index[d]	1.33	1.33	1.52	1.27	1.04	1.31	1.43
Mean chain length[e]	18.1	18.0	18.3	18.0	17.9	18.1	18.2

[a] The percentage values for the individual fatty acids contained in the cell phospholipids do not add up to 100% because only the most prevalent fatty acids are listed.

[b] Serum added to the culture medium. In each case, the serum concentration was 10%.

[c] The fatty acids are abbreviated as number of carbon atoms:number of double bonds.

[d] Average number of double bonds in each fatty acid.

[e] Average number of carbon atoms in each fatty acid.

TABLE 3

CONTENT AND COMPOSITION OF CELL PHOSPHOLIPIDS

Phospholipid	Amount	
	3T3	MDCK
Total (nanomoles per milligram protein)	342 ± 7.6	340 ± 20.9
Individual classes (% of total)		
Phosphatidylcholine	47.6 ± 1.9	42.8 ± 1.0
Phosphatidylethanolamine	23.9 ± 1.1	23.2 ± 1.9
Phosphatidylinositol	8.5 ± 0.7	8.8 ± 0.9
Phosphatidylserine	7.6 ± 1.1	7.6 ± 0.9
Others	12.4 ± 0.9	17.7 ± 2.1

with high concentrations of free fatty acid (9,31,32,35). When these modifications are produced with free fatty acids, there are changes in membrane physical properties (4,18,28,36), carrier-mediated transport (3,16,20,37), receptor binding (12,13), and other membrane-related cellular functions (14,21,23,27). It is likely that some of these effects also will occur when the phospholipid fatty acyl modifications are produced by different serum supplements. Although changes in phospholipid fatty acyl composition probably were responsible to some extent for the differences in the growth rates of the cultures (7), other serum factors must have contributed to this effect. This is evident from the fact that 3T3 cells exhibited widely different growth rates in horse and bovine calf serum even though the phospholipid fatty acid compositions were fairly similar in these cases.

In conclusion, rather extensive differences in fatty acid composition of established cell lines can occur as a result of changes in the type of serum added to the culture medium. This should be kept in mind when changes in serum are contemplated, such as during periods when there is a shortage of fetal bovine serum.

REFERENCES

1. Aveldano-DeCaldironi, M. I.; Giusto, N. M.; Bazan, N. G. Polyunsaturated fatty acids of the retina. Prog. Lipid Res. 20: 49–57; 1981.
2. Bailey, J. M.; Howard, B. V.; Dunbar, L. M.; Tillman, S. F. Control of lipid metabolism in cultured cells. Lipids 7: 125–134; 1972.
3. Balcar, V. J.; Borg, J.; Robert, J.; Mandel, P. Uptake of L-glutamate and taurine in neuroblastoma cells with altered fatty acid composition of membrane phospholipids. J. Neurochem. 38: 1678–1681; 1980.
4. Burns, C. P.; Luttennegger, D. G.; Dudley, D. T.; Buettner, G. R.; Spector, A. A. Effect of plasma membrane fatty acid composition on fluidity and methotrexate transport in L1210 murine leukemia cells. Cancer Res. 39: 1726–1732; 1979.
5. Chalvardjian, A.; Rudnicki, E. Determination of lipid phosphorus in the nanomolar range. Anal. Biochem. 36: 225–226; 1970.
6. Denning, G. M.; Figard, P. H.; Spector, A. A. Effect of fatty acid modification on prostaglandin production by cultured 3T3 cells. J. Lipid Res. 23: 584–596; 1982.
7. Doi, O.; Doi, F.; Schroeder, F.; Alberts, A. W.; Vagelos, P. R. Manipulation of fatty acid composition of membrane phospholipid and its effects on cell growth in mouse LM cells. Biochim. Biophys. Acta 509: 239–250; 1978.
8. Dunbar, L. M.; Bailey, J. M. Enzyme deletions and essential fatty acid metabolism in cultured cells. J. Biol. Chem. 250: 1152–1153.
9. Ferguson, K. A.; Glaser, M.; Bayer, W. H.; Vagelos, P. R. Alteration of fatty acid composition of LM cells by lipid supplementation and temperature. Biochemistry 14: 146–151; 1975.
10. Fine, J. B.; Sprecher, H. Unidimensional thin-layer chromatography of phospholipids on boric-acid impregnated plates. J Lipid Res. 23: 660–663; 1982.
11. Folch, J. M.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509; 1957.
12. Ginsberg, B. H.; Jabour, J.; Spector, A. A. Effect of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. Biochim. Biophys. Acta 690: 157–164; 1982.
13. Ho, W. K. K.; Cox, B. M. Reduction of opioid binding in neuroblastoma × glioma cells grown in medium containing unsaturated fatty acids. Biochim. Biophys. Acta 688: 211–217; 1982.
14. Horwitz, A. F.; Wright, A.; Ludwig, P.; Cornell, R. Interrelated lipid alterations and their influence on the proliferation and fusion of cultured myogenic cells. J. Cell Biol. 77: 334–357; 1978.
15. Hyman, B. T.; Spector, A. A. Accumulation of *n*-3 polyunsaturated fatty acids by cultured human Y79 retinoblastoma cells. J. Neurochem. 37: 60–69; 1981.
16. Im, W. B.; Deutchler, J. T.; Spector, A. A. Effects of membrane fatty acid composition on

sodium-independent phenylalanine transport in Ehrlich ascites tumor cells. Lipids 14: 1003–1008; 1979.
17. Kaduce, T. L.; Awad, A. B.; Fontenelle, L. J.; Spector, A. A. Effect of fatty acid saturation on α-aminoisobutyric acid transport in Ehrlich ascites cells. J. Biol. Chem. 252: 6624–6630; 1977.
18. King, M. E.; Spector, A. A. Effect of specific fatty acyl enrichments on membrane physical properties detected with a spin label probe. J. Biol. Chem. 253: 6493–6501; 1978.
19. Kohn, A; Gitelman, J.; Inbar, M. Unsaturated free fatty acids inactivate animal enveloped viruses. Arch. Virol. 66: 301–307; 1980.
20. Lokesh, B. R.; Wrann, M. Incorporation of palmitic acid or oleic acid into macrophage membrane lipids exerts differential effects on the function of normal mouse peritoneal macrophages. Biochim. Biophys. Acta 792: 141–148; 1984.
21. Maccecchini, M.-L.; Burger, M. M. Stimulation of lymphocytes by concanavalin A. Temperature-dependent effect of fatty acid replacements. Biochim. Biophys. Acta 469: 33–44; 1977.
22. Mackenzie, C. G.; Mackenzie, J. B.; Reiss, O. K.; Wisneski, J. A. Identification of albumin-bound fatty acids as the major factor in serum-induced lipid accumulation by cultured cells. J. Lipid Res. 11: 571–582; 1970.
23. Mahoney, E. M.; Hamill, A. L.; Scott, W. A.; Cohn, Z. A. Response of endocytosis to altered fatty acyl composition of macrophage phospholipids. Proc. Natl. Acad. Sci. USA 74: 4895–4899; 1977.
24. Meade, C. J.; Mertin, J. Fatty acids and immunity. In: Paoletti, R.; Kritchevsky, D., eds. Advances in lipid research. vol. 16. New York: Academic Press; 1978: 127–165.
25. Rosenthal, M. D.; Whitehurst, M. C. Fatty acyl Δ6 desaturation activity of cultured human endothelial cells modulation by fetal bovine serum. Biochim. Biophys. Acta 750: 490–496; 1983.
26. Schlager, S. I.; Ohanian, S. H. Modulation of tumor cell susceptibility to humoral immune killing through chemical and physical manipulation of cellular lipid and fatty acid composition. J. Immunol. 125: 1196–1200; 1980.
27. Schroit, A. J.; Gallily, R. Macrophage fatty acid composition and phagocytosis: Effect of unsaturation on cellular phagocytic activity. Immunology 36: 199–205; 1979.
28. Simon, I.; Burns, C. P.; Spector, A. A. Electron spin resonance studies on intact cells and isolated lipid droplets from fatty acid-modified L1210 murine leukemia cells. Cancer Res. 42: 2715–2721; 1982.
29. Spector, A. A.; Hoak, J. C.; Fry, G. L.; Denning, G. M.; Stoll, L. L.; Smith, J. B. Effect of fatty acid modification on prostacyclin production by cultured human endothelial cells. J. Clin. Invest. 65: 1003–1012; 1980.
30. Spector, A. A.; Kaduce, T. L.; Hoak, J. C.; Fry, G. L. Utilization of arachidonic and linoleic acids by cultured human endothelial cells. J. Clin. Invest. 68: 1003–1011; 1981.
31. Spector, A. A.; Kiser, R. E.; Denning, G. M.; Koh, S.-W. M.; DeBault, L. E. Modification of the fatty acid composition of cultured human fibroblasts. J. Lipid Res. 20: 536–547; 1979.
32. Spector, A. A.; Mathur, S. N.; Kaduce, T. L.; Hyman, B. T. Lipid nutrition and metabolism of cultured mammalian cells. Prog. Lipid Res. 19: 155–186; 1981.
33. Tinoco, J.; Babcock, I.; Hincenbergs, I.; Medwadowski, B.; Miljanich, P.; Williams, M. A. Linolenic acid deficiency. Lipids 14: 166–173; 1979.
34. Velleca, W. M.; Byrd, B. R.; Forrester, F. T. Laboratory diagnosis of mycoplasmas in sections. CDC Handbook. Washington, D.C.: U.S. Dept. of Health and Human Services; Oct. 1980. p. 25.
35. Williams, R. E.; Wisnieski, B. J.; Rittenhouse, H. G.; Fox, C. F. Utilization of fatty acid supplements by cultured animal cells. Biochemistry 13: 1969–1977; 1974.
36. Wisnieski, B. J.; Parkes, J. G.; Huang, Y. O.; Fox, C. F. Physical and physiological evidence for two phase transitions in cytoplasmic membranes of animal cells. Proc. Natl. Acad. Sci. USA 71: 4381–4385; 1974.
37. Yorek, M. A.; Strom, D. K.; Spector, A. A. Effect of membrane polyunsaturation on carrier-mediated transport in cultured retinoblastoma cells: alterations in taurine uptake. J. Neurochem. 42: 254–261; 1984.

We thank Mr. Greg R. Hanson, HyClone, Sterile Systems, Logan, UT, for supplying the serum used in this work. These studies were supported by Arteriosclerosis Specialized Center of Research Grant HL14,230 from the National Heart, Lung, and Blood Institute, National Institutes of Health.