ARTICLE

Comparison of the Metabolism of α-Linolenic Acid and Its Δ6 Desaturation Product, Stearidonic Acid, in Cultured NIH-3T3 Cells

Richard C. Cantrill*, Yung-Sheng Huang, Gregory W. Ells and **David F. Horrobin**
EFAMOL Research Institute, Kentville, Nova Scotia, Canada, B4N 4H8

The incorporation and metabolism of α-linolenic acid (18:3n-3) and its Δ6 desaturase product, stearidonic acid (18:4n-3), were compared by NIH-3T3 cells. In the presence of fetal calf serum, cells accumulated exogenously added 18:3n-3 and 18:4n-3 apparently at the expense of oleic acid (18:1n-9). Both 18:3n-3 and 18:4n-3 were elongated and desaturated to eicosatetraenoic acid (20:4n-3), eicosapentaenoic acid (20:5n-3) and docosapentaenoic acid (22:5n-3), but not to docosahexaenoic acid (22:6n-3), and were incorporated into phospholipids and triacylglycerols. Over a 4-d period, the growth of NIH-3T3 cells was slightly stimulated in the presence of 18:3n-3 (20 μg/mL) but was strongly inhibited in the presence of 18:4n-3 at the same concentration. This inhibition may be caused by enhanced lipid peroxidation as a result of the high levels of 18:4n-3 present. *Lipids 28,* **163-166 (1993).**

In recent years an increased dietary intake of fish oils, rich in long-chain n-3 fatty acids, has been vigorously promoted for its possible medical benefits. For instance, eicosapentaenoic acid (20:5n-3) may reduce the risk of thrombosis and, consequently, the risk of coronary disease (1,2). However, there is increasing evidence that excessive fish oil intake may induce adverse side effects (3,4). Some researchers have suggested that increasing the intake of α-linolenic acid (18:3n-3), the precursor of long-chain n-3 fatty acids, may provide the same beneficial effects but minimize the adverse effects of fish oil supplementation (5,6). Unfortunately, the activity of Δ6 desaturase in humans is relatively low (7), thus limiting the formation of long-chain n-3 fatty acids and, consequently, their potential beneficial effects.

Stearidonic acid (18:4n-3), the Δ6 desaturation product of 18:3n-3, is found in small quantities in borage and blackcurrant oils (8-10) and certain fish oils (for a review, see ref. 11). It has previously been shown in experimental animals that stearidonic acid is a more effective dietary supplement than α-linolenic acid in the formation of long-chain n-3 fatty acids (12). In other words, supplementing the diet with stearidonic acid could compensate for the low activity of Δ6 desaturase and low availability of its products.

To date few studies have compared the metabolism of stearidonic acid and α-linolenic acid in cultured cells. Previous studies in our laboratory have shown that administration of certain n-6 polyunsaturated fatty acids (PUFA) in the concentration range of 5-60 μg/mL kills many tumor cell lines in culture (13-15). We (16) and others (17) have also shown that 18:4n-3 was toxic to tumor cells in culture; however, its effect on a normal cell line has not been reported. In the present study, stearidonic acid and its direct precursor, α-linolenic acid, were added individually, to cell culture medium, and their effects on NIH-3T3 cells, a nonmalignant cell line, were compared. Cell growth was determined by cell counting, and the extent of incorporation and modification of 18:3n-3 and 18:4n-3 was determined in lipid extracts of the cells.

*To whom correspondence should be addressed at the EFAMOL Research Institute, P.O. Box 818, Kentville, Nova Scotia, Canada, B4N 4H8.

Abbreviations: Chol, cholesterol; DMEM, Dulbecco's modified essential medium; HPLC, high-performance liquid chromatography; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI + PS, phosphatidylinositol and phosphatidylserine; PUFA, polyunsaturated fatty acids; TG, triacylglycerol.

MATERIALS AND METHODS

Cell culture. NIH-3T3 cells, obtained from Dr. R. Bassin (NIH, Bethesda, MD), were maintained in Dulbecco's modified essential medium (DMEM) (Flow Labs, Mississauga, Ontario, Canada) containing 10% fetal calf serum (Flow Labs), without the addition of antibiotics. The protocol for the examination of the effects of 18:3n-3 and 18:4n-3 on cell growth has been described previously (18). Cells were seeded in 24-well plates at a density of 10^4 cells per well (0.5 mL) and allowed to attach. Twenty-four hours later (Day 0), the medium was replaced with fresh medium containing 5% fetal calf serum (total fatty acid content = 12.5 μg/mL medium; major fatty acids in percent: 16:0, 21; 16:1, 5; 18:0, 12; 18:1, 24; 18:2n-6, 6; 20:3n-6, 3; 20:4n-6, 11; 20:5n-3, 1; 22:4n-6, 1; 22:5n-6, 2; 22:5n-3, 3; 22:6n-3, 4) and either 18:3n-3 or 18:4n-3. Solutions of each fatty acid in ethanol (40 mg/mL) were diluted with medium to give final concentrations between 5 and 30 μg/mL. The final ethanol concentration was maintained at 0.2%. Control wells received fresh medium containing 0.2% ethanol. Cells were observed daily by phase contrast microscopy. After an additional 4 d in culture, cells were detached with trypsin and counted in a haemocytometer counting chamber. The trypan blue exclusion technique was used to determine the number of dead cells (19). Some experiments were carried out in the presence of ferrous ions ($FeCl_2.4H_2O$ 10 μg/mL) or dl-α-tocopherol (Sigma, St. Louis, MO; 10 μM).

Determination of fatty acid profiles. For lipid analysis, cells were seeded in 5 mL medium at a density of 10^5 cells per 6-cm Petri dish and supplemented with either 18:3n-3 or 18:4n-3 according to the protocol described above. After 3 d cells were released from the dish with trypsin, pelleted at 900 × *g* and resuspended in phosphate buffered saline (pH 7.4, composition as in ref. 20). For fatty acid quantitation, 17:0 was added to each sample as internal standard. Lipids were extracted by the method of Folch *et al.* (21), and the lipid class distribution was determined by high-performance liquid chromatography (HPLC) (22). Data were expressed as area percentage and were taken directly from the HPLC/mass detector analysis. Different lipid classes were expressed as a ratio which was calculated from the area percentage data for individual samples. For fatty acid analysis, phospholipids and triacylglycerols (TGs) were first separated by thin-layer chromatography (23). After transmethylation of lipid extracts (24), the fatty acid composition of the growth

medium, cells and phospholipid and TG fractions was determined by gas-liquid chromatography as described elsewhere (12).

All data are presented as mean ± SD (n = 4). Statistical analysis was performed using one-way analysis of variance and the Student's *t*-test using the SPSS-PC+ software package (SPSS Inc., Chicago, IL).

RESULTS

Effects of 18:3n-3 and 18:4n-3 on cell growth. Supplementation with either fatty acid (20 μg/mL) led to the appearance of lipid droplets within 24 h. The number of droplets decreased as time progressed. This observation has also been made when NIH-3T3 cells were incubated with other exogenous fatty acids (16:0, 18:2n-6, 18:3n-6 and 20:4n-6; Cantrill, Ells and Horrobin, unpublished data). Phase contrast microscopy revealed no other changes in cell morphology in either control or supplemented media. Cell number was not affected by either fatty acid during the first two days after supplementation. Thereafter, cells grown in 18:4n-3 supplemented medium ceased to proliferate, whereas cells grown in the control medium and those treated with 18:3n-3 continued to divide (Fig. 1). On Day 4 the number of cells in the 18:3n-3 supplemented medium was greater than in the control medium.

The effects of 18:3n-3 and 18:4n-3 supplementation at different concentrations are shown in Figure 2. On Day 4, 18:4n-3 at concentrations greater than 10 μg/mL had significantly suppressed the proliferation of NIH-3T3 cells and few viable cells remained at concentrations above 25 μg/mL. On the other hand, 18:3n-3 caused a 25–50% increase in cell number above control levels. The effect of 18:4n-3 was exacerbated by the addition of ferrous ions ($FeCl_2.4H_2O$ 10 μg/mL) and reduced by the simultaneous addition of dl-α-tocopherol (10 μM) (Fig. 2).

Cellular lipid distribution. To compare the effect of 18:3n-3 and 18:4n-3 supplementation on cellular lipid class distribution, cells were harvested, and analyses were performed on Day 3, before any cytotoxic effects were evident. Table 1 shows that the presence of either fatty acid caused a marked increase in the relative amount of TG and phosphatidylethanolamine (PE) and a decrease in the proportions of free cholesterol (chol) and phosphatidylcholine (PC) in total cellular lipids. The ratios of different lipid classes were calculated in order to determine if these relative changes were solely a reflection of TG formation or were more complex. Cells treated with 18:4n-3, as compared with 18:3n-3, had a 40% increase in the chol/PC ratio, a 20% increase in the PE/PC ratio and a 33% increase in the TG/chol ratio (Table 1). These results indicate that the two fatty acids have different effects on lipid class distribution.

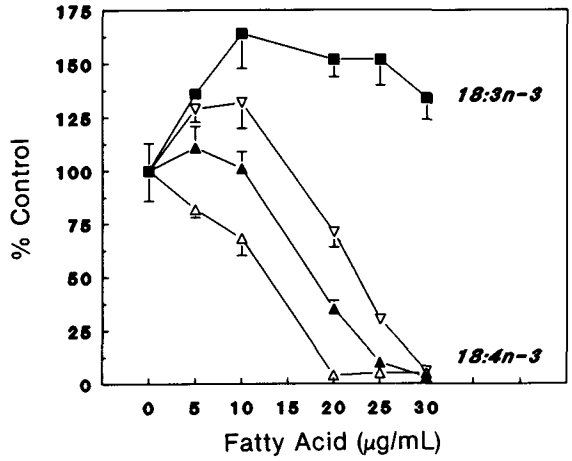

FIG. 2. Concentration-dependent effect of 18:3n-3 and 18:4n-3 on NIH-3T3 cells. Cells were cultured for four days in the presence of different concentrations of either 18:3n-3 (■) or 18:4n-3 (▲) between 0 and 30 μg/mL. 18:4n-3 treated cells were also grown in the presence of either ferrous ions ($FeCl_2.4H_2O$ 10 μg/mL) (△) or dl-α-tocopherol (10 μM) (▽). Data are presented as % control (total cell count at 0 μg/mL fatty acid) ± SD (n = 4).

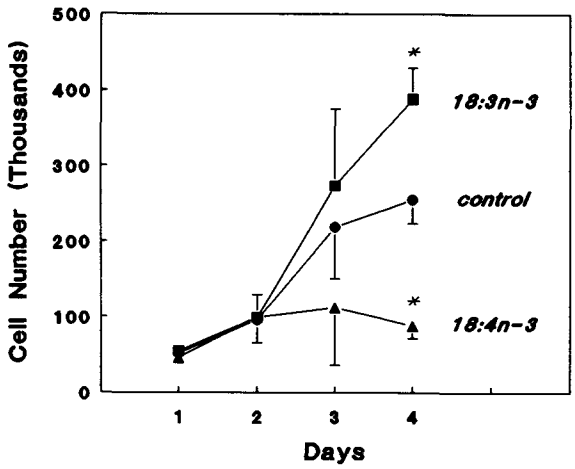

FIG. 1. Time-dependent effect of 18:3n-3 and 18:4n-3 on NIH-3T3 cells. Cells were cultured for four days in control medium (●), or in the presence of 20 μg/mL of either 18:3n-3 (■) or 18:4n-3 (▲). Data are presented as mean total cell count ± SD (n = 4). *Cell counts significantly different ($P < 0.05$) from control values on the same day.

TABLE 1

Lipid Class Distribution of NIH-3T3 Cells Treated for Three Days with 18:3n-3 or 18:4n-3[a]

	Control	18:3n-3	18:4n-3
Area %			
TG[b]	trace[c]	10.7 ± 1.0	15.9 ± 3.1[d]
Chol	14.5	10.5 ± 0.5	11.7 ± 1.0
PE	19.1	23.1 ± 0.9	22.2 ± 0.5
PI + PS	1.6	2.3 ± 0.1	0.9 ± 0.1[d]
PC	60.7	46.0 ± 0.6	36.8 ± 3.2[d]
SPM	trace	3.7 ± 0.2	3.6 ± 2.2
Ratio			
Chol/PC	0.24	0.23 ± 0.02	0.32 ± 0.04[d]
Chol/PE	0.76	0.45 ± 0.01	0.53 ± 0.04[d]
PE/PC	0.31	0.50 ± 0.04	0.60 ± 0.05
TG/Chol	—	1.03 ± 0.12	1.37 ± 0.35

[a]Results are expressed as area % ± SD (n = 4). For abbreviations, see footnote to title.

[b]May contain trace amounts of cholesterol esters.

[c]Present at less than 1%.

[d]Significantly different from 18:3n-3 treated cells ($P < 0.05$).

TABLE 2

Fatty Acid Composition of NIH-3T3 Cells Grown in Culture with Either 18:3n-3 or 18:4n-3 at a Concentration of 20 μg/mL[a]

Fatty acid	Day 1			Day 3		
	Control	18:3n-3	18:4n-3	Control	18:3n-3	18:4n-3
Total	32.2 ± 10.7	48.2 ± 14.7	32.2 ± 11.4	19.8 ± 2.3	30.8 ± 7.6	65.2 ± 9.7
18:3n-3	trace[b]	21.2 ± 6.4[c]	0.1 ± 0.0[d]	trace	5.2 ± 1.1[c]	0.3 ± 0.1[d]
18:4n-3	0.1 ± 0.1	1.5 ± 0.5[c]	10.7 ± 4.3[c,d]	0.2 ± 0.0	1.1 ± 0.2[c]	17.3 ± 2.1[c,d]
20:4n-3	0.1 ± 0.0	0.8 ± 0.3[c]	1.3 ± 0.5[c]	trace	1.2 ± 0.3[c]	6.7 ± 1.1[c,d]
20:5n-3	0.2 ± 0.1	1.2 ± 0.4[c]	1.3 ± 0.6[c]	0.1 ± 0.0	4.2 ± 1.0[c]	4.9 ± 1.1[c]
22:5n-3	1.3 ± 0.5	1.3 ± 0.4	0.9 ± 0.5	0.2 ± 0.0	1.4 ± 0.3[c]	1.4 ± 0.3[c]
22:6n-3	1.3 ± 0.5	1.1 ± 0.3	1.3 ± 0.9	0.3 ± 0.0	0.3 ± 0.1	1.5 ± 0.5[c,d]
Total n-3	3.1	27.1	15.6	0.8	13.5	32.3
% Total	9.5%	56%	48%	3.9%	44%	50%
Total n-6	7.0 ± 2.3	5.0 ± 1.6	3.1 ± 1.3	2.5	2.1 ± 0.5	5.2 ± 0.2
18:1n-9	7.2 ± 2.5	4.0 ± 1.2	3.5 ± 0.9	7.4	3.1 ± 0.7	6.5 ± 0.8

[a]Results are expressed as μg/10^6 cells, mean ± SD (n = 4).
[b]Indicates present in amounts below 0.1 μg/10^6 cells.
[c]Significantly different from control values ($P < 0.05$).
[d]Significantly different from 18:3n-3 treated values ($P < 0.05$).

Cellular fatty acid composition. Incubation with either 18:4n-3 or 18:3n-3 significantly increased the amount of n-3 fatty acids in total cellular lipid extracts. The n-3 fatty acids increased at the expense of monounsaturated, especially 18:1n-9, and n-6 fatty acids (Table 2). On Day 1 only modest amounts of elongation and desaturation products of either 18:3n-3 or 18:4n-3 were present, and no increases in the amounts of 22:5n-3 and 22:6n-3 were seen. By Day 3, the amount of the products was increased above Day 1 values. The major metabolite of 18:3n-3 was 20:5n-3, and the major metabolites of 18:4n-3 were 20:4n-3 and 20:5n-3. The amount of 22:6n-3 was increased above control values only in 18:4n-3 supplemented cells (Table 2).

The cellular fatty acid content (μg/10^6 cells) in 18:3n-3 treated cells was slightly higher than in control or in 18:4n-3 treated cells on Day 1 (Table 2). This resulted from an increase in total cellular n-3 fatty acids. In 18:3n-3 and 18:4n-3 treated cells, the levels of n-3 fatty acids were 27.1 and 15.6 μg/10^6 cells, respectively, and constituted approximately 50% of the total fatty acids. This was more than a fourfold increase above the levels present in control cells.

On Day 3, the total fatty acid content of both control and 18:3n-3 treated cells was reduced by 30% with respect to Day 1 values. However, the fatty acid content of 18:3n-3 treated cells remained 50% higher than that of control cells on Day 3. The fatty acid content of 18:4n-3 treated cells on Day 3 was twice that measured on Day 1. This value was also 2- and 3-fold higher than that of the 18:3n-3 treated and of the control cells, respectively, on Day 3. At this time, the levels of n-3 fatty acids were 4% of the total fatty acid content in control cells and 44 and 50% in 18:3n-3 and 18:4n-3 treated cells, respectively.

Table 3 shows the distribution of 18:3n-3 and 18:4n-3 and their metabolites in the TG and phospholipid fractions on Day 3. In the TG fraction, most of the n-3 fatty acids in the 18:3n-3 treated cells were recovered as 18:3n-3. However, a significant proportion of 18:4n-3 had been elongated to 20:4n-3 and subsequently desaturated to 20:5n-3 in the 18:4n-3 treated cells. In the phospholipid fraction, 20:5n-3 was the major product in both 18:3n-3 and 18:4n-3 treated cells, and a substantial amount of 20:4n-3 was also observed in the 18:4n-3 treated cells. The results also show that the amount of 22:5n-3 or of 22:6n-3 did not depend on the precursor fatty acid.

TABLE 3

Percent Distribution of n-3 Fatty Acids in Triacylglycerol and Phospholipid Fractions of NIH-3T3 Cells Incubated for Three Days with Either 18:3n-3 or 18:4n-3 (20 μg/mL)[a]

Fatty acid	Control	18:3n-3	18:4n-3
Triacylglycerols			
18:3n-3	0.2 ± 0.1	33.3 ± 1.3[c]	0.5 ± 0.1[d]
18:4n-3	0.5 ± 0.3	5.1 ± 0.5[c]	17.4 ± 6.3[c,d]
20:4n-3	trace[b]	7.6 ± 0.3[c]	27.9 ± 1.0[c,d]
20:5n-3	trace	8.0 ± 0.4[c]	13.6 ± 1.9[c,d]
22:5n-3	0.1 ± 0.1	3.8 ± 0.3[c]	4.1 ± 1.4[c]
22:6n-3	0.2 ± 0.1	1.1 ± 0.1[c]	0.8 ± 0.2[c]
Phospholipids			
18:3n-3	0.3 ± 0.1	22.4 ± 0.2[c]	1.3 ± 0.3[c,d]
18:4n-3	trace	3.2 ± 0.3[c]	9.9 ± 3.3[c,d]
20:4n-3	trace	3.1 ± 0.1[c]	10.7 ± 0.6[c,d]
20:5n-3	0.4 ± 0.1	11.9 ± 0.6[c]	15.2 ± 1.8[c,d]
22:5n-3	1.0 ± 0.1	3.0 ± 0.1[c]	4.0 ± 1.0[c]
22:6n-3	1.3 ± 0.2	1.1 ± 0.1	1.5 ± 0.2

[a]Results are expressed as mean ± SD (n = 4).
[b]Present at less than 0.1%.
[c]Significantly different from control values ($P < 0.05$).
[d]Significantly different from the 18:3n-3 values ($P < 0.05$).

DISCUSSION

Supplementation of growth medium with either 18:3n-3 or 18:4n-3 increased the quantities of n-3 fatty acids in NIH-3T3 cells. As shown in Table 2, however, the total fatty acid content on Day 3 was 30% lower than on Day 1 in both control and 18:3n-3 treated cells, whereas it was increased 2-fold in 18:4n-3 treated cells. The explanation for these differences is probably related to the rate of cell proliferation, since the number of 18:4n-3 treated cells had only doubled since Day 1, whereas the number of 18:3n-3 treated cells had increased 6-fold.

Treatment with 18:3n-3 increased the levels of the post Δ6 desaturase n-3 fatty acids (18:4n-3, 20:4n-3, 20:5n-3 and 22:5n-3) indicating that NIH-3T3 cells have an active Δ6 desaturase system (Table 2). In 18:4n-3 treated cells, there was an increase in 20:4n-3 levels but the level of 20:5n-3 was not affected. The data suggest that Δ5 desaturase activity was low in these cells and might have limited the production of 20:5n-3. Following either treatment, the amounts of 22:5n-3 and 22:6n-3 were similar on both Day 1 and Day 3.

It is also possible that the metabolism of n-3 fatty acids is not solely regulated by the activity of the desaturation and elongation enzymes but also by their incorporation into phospholipid and TG fractions. It has been proposed recently (25) that the distribution and metabolism of n-6 fatty acids is a consequence of the availability of acceptor molecules (PUFAs are predominantly found in the 2-position of phospholipids). There also exists the possibility of competition between n-3 fatty acids of different chain-length and degree of unsaturation for esterification into different positions of TG and phospholipid molcules.

An increased supply of n-3 fatty acids increased their incorporation into phospholipids and also led to the formation of TGs (Table 1). Acylation into TGs and the subsequent formation of intracellular perinuclear lipid droplets is evidence of another cellular response to excess PUFA intake. The formation of these lipid droplets was probably responsible for the changes in the lipid class ratios (Table 1). These changes could not be attributed solely to the increase in TG content since the proportions of different lipids were not reduced to the same extent. Thus, it is likely that the formation of the lipid droplet boundary membrane led to the alterations in the cholesterol/PC and the PE/PC ratios (Table 1). These shifts in the chol/phospholipid ratios may have also induced the incorporation of more PUFA into phospholipids.

If the incorporation of PUFA into phospholipid is restricted by the number of sites on suitable acceptor molecules, then excess fatty acid esterified into neutral lipids may be subsequently incorporated into phospholipid as cell number increases (26). In 18:3n-3 treated cells, the formation of long-chain metabolites and their accumulation in neutral lipid stores was lower than in 18:4n-3 treated cells (Table 3). This may have served to reduce the accumulation of substrates for lipid peroxidation since it has been shown previously that fatty acids with three and more double bonds are cytotoxic to many tumor cell lines in culture (14). This process is thought to be caused by the formation of lipid peroxides (27) since the cytotoxic effect of post Δ6 desaturase n-6 fatty acids cannot be prevented by certain cyclooxygenase or prostaglandin synthetase inhibitors (28). However, cell death may be accelerated by the addition of ferrous or copper ions and blocked by vitamin E and other antioxidants (18). Our observations support the involvement of lipid peroxidation in the lethal process (Fig. 2). The provision of 18:4n-3 to cells leads to the accumulation of substrates for lipid peroxidation, a process which is likely to cause cell death.

ACKNOWLEDGMENTS

The authors thank Valerie Simmons and Rick Smith for technical assistance.

REFERENCES

1. Weiner, B.H. Ockone, I.S., Levine, P.H., Cuénod, H.F., Fisher, M., Johnson, B.F., Daoud, A.S., Jarmolych, J., Hosmer, D., Johnson, M.H., Natale, A., Vandreuil, C., and Hoogasian, J.J. (1986) *N. Engl. J. Med. 315,* 841-846.
2. Dyerberg, J., Bang, H.O., Stoffersen, E., Moncada, S., and Vane, J.R. (1978) *Lancet ii,* 117-119.
3. Sullivan, D.R., Sanders, D.A.B., Trayner, I.M., and Thompson, G.R. (1986) *Atherosclerosis 61,* 129-134.
4. Thiery, J., and Seidal, D. (1987) *Atherosclerosis 63,* 53-58.
5. Hunter, J.E. (1987) *N. Engl. J. Med. 316,* 626.
6. Simopoulos, A.P., and Salem, N., Jr. (1987) *N. Engl. J. Med. 315,* 833 (letter).
7. Horrobin, D.F., Manku, M.S., and Huang, Y.-S. (1984) *Biomed. Biochim. Acta 43,* S114-121.
8. Hörhammer, L., Wagner, N., and König, H. (1961) *Arzneim.-Forsch. 14,* 34-40.
9. Wagner, N., and König, H. (1963) *Biochem. Z. 339,* 212-218.
10. Traitler, H., Winter, H., Richli, U., and Ingenbleek, Y. (1984) *Lipids 19,* 923-928.
11. Ackman, R.G. (1982) in *Nutritional Evaluation Of Long-chain Fatty Acids in Fish Oil* (Barlow, S.M. and Stansby, M.E., eds.) pp. 25-88, Academic Press, London.
12. Huang, Y.-S., Smith, R., Redden, P.R., Cantrill, R.C., and Horrobin, D.F. (1991) *Biochim. Biophys. Acta 1082,* 319-327.
13. Horrobin, D.F. (1990) in *Omega-6 Essential Fatty Acids: Pathophysiology and Roles in Clinical Medicine* (Horrobin, D.F., ed.), pp. 351-377, Wiley-Liss, New York.
14. Begin, M.E., Ells, G., Das, U.N., and Horrobin, D.F. (1986) *J. Natl. Cancer Inst. 77,* 1053-1062.
15. Chow, S.C., Sisfontes, L., Björkhem, I., and Jondal, M. (1989) *Lipids 24,* 700-704.
16. Cantrill, R.C., Huang, Y.-S., Ells, G., DeAntueno, R., and Horrobin, D.F. (1992) *INFORM 3,* 517-518.
17. Cornelius, A.S., Yerram, N.R., Kratz, D.A., and Spector, A.A. (1991) *Cancer Res. 51,* 6025-6030.
18. Begin, M.E., Ells, G., and Horrobin, D.F. (1988) *J. Natl. Cancer Inst. 80,* 188-194.
19. Freshney, R.I. (1983) in *Culture of Animal Cells: A Manual of Basic Technique,* pp. 207-209, Alan R. Liss Inc., New York.
20. Heithier, H., Ward, L.D., Cantrill, R.C., Klein, H., Im, M.-J., Pollack, G., Freeman, B., Schiltz, E., Peters, R., and Helmreich, E.J.M. (1988) *Biochim. Biophys. Acta 971,* 298-306.
21. Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957) *J. Biol. Chem. 226,* 497-509.
22. Redden, P., and Huang, Y.-S. (1991) *J. Chromatog. 567,* 21-27.
23. Manku, M.S., Horrobin, D.F., Huang, Y.-S., and Morse, N. (1983) *Lipids 18,* 906-908.
24. Morrison, W.R., and Smith, L.M. (1964) *J. Lipid Res. 5,* 600-608.
25. Huang, Y.-S., Redden, P.R., DeAntueno, R.J., Cantrill, R.C., and Horrobin, D.F. (1992) *Third Intl. Conf. on Essential Fatty Acids and Prostaglandins,* abstract 106.
26. Spector, A.A., Mathur, S.N., Kaduce, T.L., and Hyman, B.T. (1981) *Prog. Lipid Res. 19,* 155-186.
27. Das, U.N., Begin, M.E., Ells, G., Huang, Y.-S., and Horrobin, D.F. (1987) *Biochem. Biophys. Res. Commun. 145,* 15-24.
28. Botha, J.H., Robinson, K.M., and Leary, W.P. (1985) *Prostaglandins Leukotr. Med. 19,* 63-67.

[Received July 25, 1992, and in final revised form December 21, 1992; Revision accepted December 26, 1992]