Involvement of triacylglycerol in the metabolism of fatty acids by cultured neuroblastoma and glioma cells

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Abstract The metabolism (chain elongation, desaturation, and incorporation into complex lipids) of thirteen different radiolabeled fatty acids and acetate was examined in N1E-115 neuroblastoma and C-6 glioma cell lines in culture. During 6-hr incubations, all fatty acids were extensively (14–80%) esterified to complex lipids, mainly choline phosphoglycerides and triacylglycerol. With trienoic and tetraenoic substrates, inositol and ethanolamine phosphoglycerides also contained up to 30% of the labeled fatty acids; plasmalogen contained up to half of the label in the ethanolamine phosphoglyceride fraction of neuroblastoma cells. Chain elongation and Δ9, Δ6, and Δ5 desaturation occurred in both cell lines; Δ4 desaturation was not observed. Seemingly anomalous utilization of arachidic acid and some selectivity based on the geometric configuration of double bonds was observed. These studies indicate that these cell lines are capable of modulating cellular membrane composition by a combination of selective exclusion and removal of inappropriate acyl chains and of modification of other acyl chains by desaturation and chain elongation. The time courses and patterns of modification and incorporation of exogenous substrates into phospholipids and triacylglycerol suggest that exogenous unsaturated fatty acid may be incorporated into triacylglycerol and later released for further metabolism and incorporation into phospholipids. This supports a role for triacylglycerol in the synthesis of membrane complex lipids in cell lines derived from neural tissue.—**Cook, H. W., J. T. R. Clarke, and M. W. Spence.** Involvement of triacylglycerol in the metabolism of fatty acids by cultured neuroblastoma and glioma cells. *J. Lipid Res.* 1982. **23:** 1292–1300.

Supplementary key words chain elongation • desaturation • phospholipids

The activities of membrane-associated enzymes and transport of metabolites across membranes are affected by their lipid environment (1, 2). Variations in the fatty acid profile of membrane phospholipids play a role in such modulation of membrane properties. Knowledge of how the fatty acid composition is established and maintained is, therefore, essential for understanding many membrane-related properties.

Cell lines of neural origin, such as neuroblastoma and glioma cells, maintained in long-term culture, provide valuable tools for investigation of membrane-related properties. While these tumor cell lines have limitations, in that they do not exhibit all the properties of normal differentiated cells, they do exhibit some important characteristics relevant to the intact nervous system. Further, tissue culture techniques allow investigations of a single cell-type in a controlled environment. For example, the acyl chain composition of neuroblastoma cells can be altered by changes in fatty acid composition and serum content of their growth medium (3, 4). Moreover, these cells can metabolize essential fatty acid to more unsaturated or longer chain acids and incorporate these modified acyl chains into membrane phospholipids (3–5).

Membrane phospholipids of brain tumors (6–8) and of cultured cell lines of neural-tumor origin (4) have fatty acyl compositions that differ from corresponding normal tissues; in particular, tumor cells contain lower levels of long chain polyunsaturated acids with 20 and 22 carbon atoms. It has been suggested that incorporation of the polyunsaturated acids may be limited by decreased desaturase activities (3, 5, 9–11) and that capacity for desaturation at the Δ4 position may be totally absent in neuroblastoma cells.

To assess the ability of neural cells in culture to modulate membrane lipid composition, we examined the relative incorporation and metabolism of 14 different radiolabeled fatty acids or precursor molecules in neuroblastoma N1E-115 and C-6 glioma cell lines in culture. The enzymatic activities of these cell lines provide for the exclusion and removal of inappropriate acyl chains and the modulation of cellular membrane composition by a combination of desaturation and chain elongation reactions. In addition, a role for triacylglycerol as an intermediate in exogenous unsaturated fatty acid metabolism is indicated.

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

MATERIALS AND METHODS

Materials

All chemicals and reagents used were of the finest available grade obtained from various commercial suppliers. 1-^{14}C-Labeled fatty acids were purchased as follows: acetyl-CoA (53 mCi/mmol), palmitic acid (16:0, 50 mCi/mmol), stearoyl-CoA (18:0-CoA, 60 mCi/mmol), stearic acid (18:0, 52 mCi/mmol), oleic acid (18:1(n-9), 52 mCi/mmol), linoleic acid (18:2(n-6), 50 mCi/mmol), linolenic acid (18:3(n-3), 52 mCi/mmol), eicosatrienoic acid (20:3(n-6), 57 mCi/mmol), and arachidonic acid (20:4(n-6), 56 mCi/mmol) from NEN Canada, Lachine, PQ; palmitoleic acid (16:1(n-7), 57 mCi/mmol) and arachidic acid (20:0, 56 mCi/mmol) from Applied Sciences Laboratories, State College, PA; vaccenic acid (18:1(n-7), 50 mCi/mmol), elaidic acid (t-18:1(n-9), 56 mCi/mmol), and linoelaidic acid (t-18:2(n-6), 15 mCi/mmol) from Amersham Corporation, Oakville, ON, by special order. The purity of these acids, determined by several analytical procedures as described previously (12), was >97%. Nonradioactive fatty acids and lipid standards were purchased from Serdary Research Labs., St. Catherines, ON. All solvents were HPLC grade from Fisher Scientific, Dartmouth, NS.

Neuroblastoma and glioma cultures

N1E-115 neuroblastoma and C-6 glioma cell lines were maintained in 150-cm^2 flasks (Corning Glass Works, Corning, NY) in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum (Gibco Canada Ltd., Burlington, ON) and penicillin–streptomycin solution (100 units and 100 g/ml, respectively). Cells were maintained at 37°C in a humidified atmosphere of 95% air–5% CO_2 and subcultured every 5–7 days. For incorporation and metabolism studies, cells were transferred to 25-cm^2 flasks at a density of 0.5–1.0 × 10^6 cells per flask in 5 ml of medium. After 72 hr, the medium was removed and replaced with 2.5 ml of DMEM without fetal calf serum. After 24 hr, radioactive fatty acid, suspended by sonication at 37°C in sterile 5% bovine serum albumin, was added in a 100-μl volume to give a final fatty acid concentration of 100 μM and 2 × 10^6 dpm of ^{14}C per dish (unless otherwise specified). Cells were incubated for 6 hr at 37°C unless otherwise indicated.

Extraction of lipids

After incubation, the medium was decanted and the cells were harvested following a 1-min incubation in 0.5 ml of 0.25% trypsin to release the cells. Fetal calf serum (0.5 ml) was added to inhibit the trypsin and the dishes were washed four times with 2 ml of phosphate-buffered saline. Comparisons with three other methods of cell harvesting showed that lipid labeling profiles from cells harvested by trypsinization were indistinguishable from those of cells harvested by releasing cells by repeated flushing with cold buffer or by freezing the cells in situ followed by scraping into ice-cold buffer and lyophilization. Moreover they were little different from those denatured with 30% trichloroacetic acid prior to scraping. Thus, the routine trypsinization procedure appeared not to alter the true profile of fatty acid incorporation into phospholipid and neutral lipid. After a final centrifugation at 2000 *g* for 10 min, the cell pellet was suspended in phosphate-buffered saline, and aliquots were taken for cell counts and protein analysis. The remainder was centrifuged, the supernatant was combined with the original medium, and an aliquot of the mixture was counted for total radioactivity.

The cell pellet was suspended in 8 ml of chloroform–methanol 2:1 and left overnight at 4°C under N_2. After adding 1.6 ml of 0.1 N KCl, the mixture was centrifuged and the upper phase was removed and discarded. The organic lower phase was filtered and dried under N_2. The lipid extract was suspended in chloroform–methanol 19:1 and an aliquot was counted in 5 ml of Beckman HP scintillation cocktail in a Searle Delta 300 counter.

Analysis of the lipid extract

The lipid extract was separated into neutral lipid, glycolipid, and phospholipid fractions by silicic acid column chromatography (13). Resolution of neutral lipids (13), phospholipids (13), glycolipids (14), plasmalogen (15), and methyl esters (16) was achieved by thin-layer chromatography. Methyl esters were prepared by treatment of the lipids with 10% BF_3 in methanol (17). Radio–GLC of the methyl esters was carried out on 1.5 m × 2 mm (ID) glass column packed with 15% Silar 9CP and a radioactivity-flow monitor as described (18).

Triacylglycerol concentration (19) and phospholipid phosphorus (20) were determined on lipid fractions isolated by preparative TLC or lipid extracts from 10^7 cells. Protein was determined by the method of Lowry et al. (21), with bovine serum albumin as standard.

RESULTS

Total lipid, phospholipid, and triacylglycerol contents were slightly less for neuroblastoma (0.17 ± 0.01 mg lipid/mg protein; 103 ± 10 nmol phospholipid/mg protein; 76 ± 19 nmol triacylglycerol/mg protein; respectively, n = 3) than for glioma cells (0.21 ± 0.02 mg/mg protein; 127 ± 13 nmol/mg; 79 nmol/mg, re-

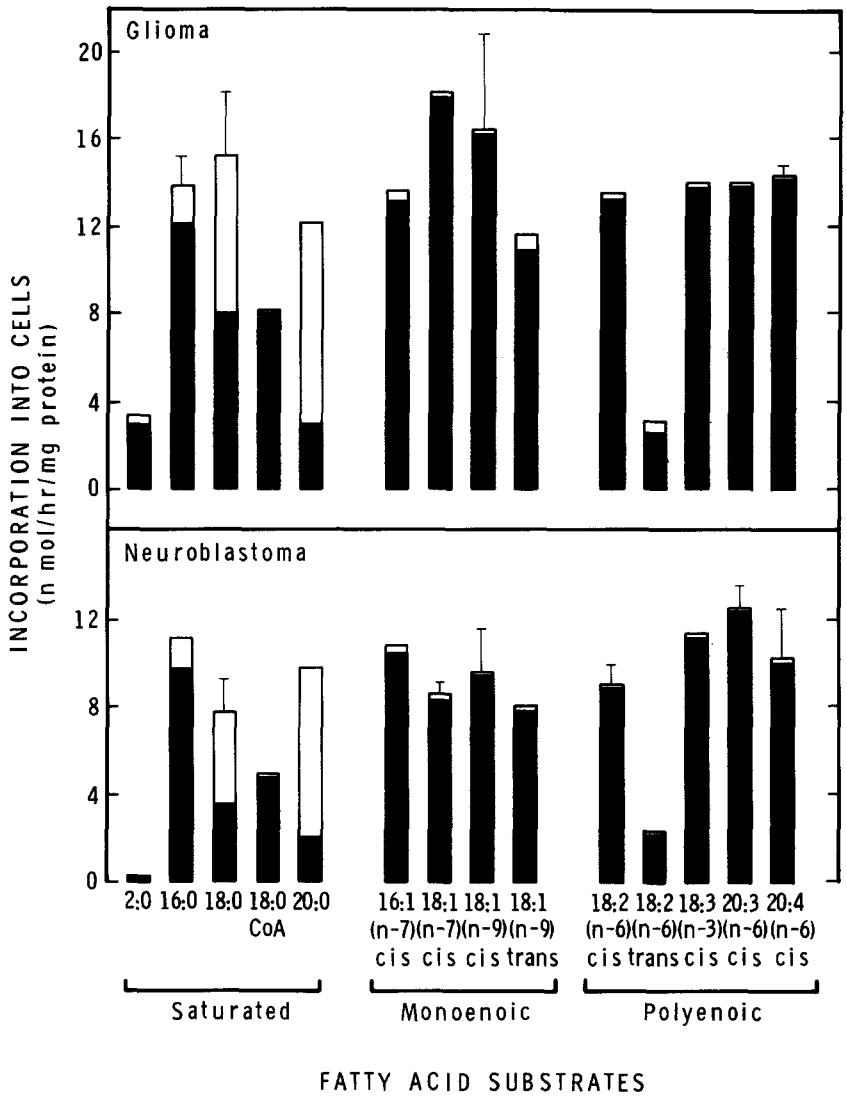

Fig. 1. Incorporation of fatty acids and acetate into glioma and neuroblastoma cells during 6-hr incubations. All substrates were added in 5% BSA to give an initial medium concentration of 100 μM except for 18:0-CoA and sodium acetate, which were added as aqueous solutions. Total bar represents total cell-associated label from fatty acid; the shaded portion represents esterified fatty acyl chains. The brackets indicate standard deviations (N = 6). Bars without brackets represent the mean of duplicate determinations. Amount of incorporation (nmol/hr per mg protein) was derived from the ^{14}C content of the cells following incubation with the 1-^{14}C-labeled fatty acid or precursor molecules.

spectively). Choline phosphoglyceride accounted for 52 ± 4% and ethanolamine phosphoglyceride for 22 ± 3% of the total phospholipid of both cell lines. Neuroblastoma contained 2–3 times more inositol phosphoglyceride and sphingomyelin than glioma but the amounts of the other major phospholipids were similar for the two cell lines.

Incorporation of labeled fatty acids into complex lipid

Combined recoveries of radioactivity in the cell extract and culture medium were >96% in all cases. Incorporation of label into nonlipid metabolites was not detected. Radioisotope in the growth medium and cell washes (10–45% of the total label) occurred entirely as unaltered free fatty acid. Thus, there was no evidence of loss of labeled complex lipid from the cultured cells either by exchange or cell death and dissolution.

Fig. 1 shows that incorporation of exogenous acetate into complex lipids by neuroblastoma was negligible; glioma cells incorporated up to 15% of acetate label into complex lipid. All fatty acids of 16 carbons or greater were readily incorporated into complex lipids of both cell lines. Total incorporation and esterification of the monoenoic and polyenoic *cis*-acids were similar. In contrast, *trans*-fatty acids, particularly *trans,trans*-18:2, were

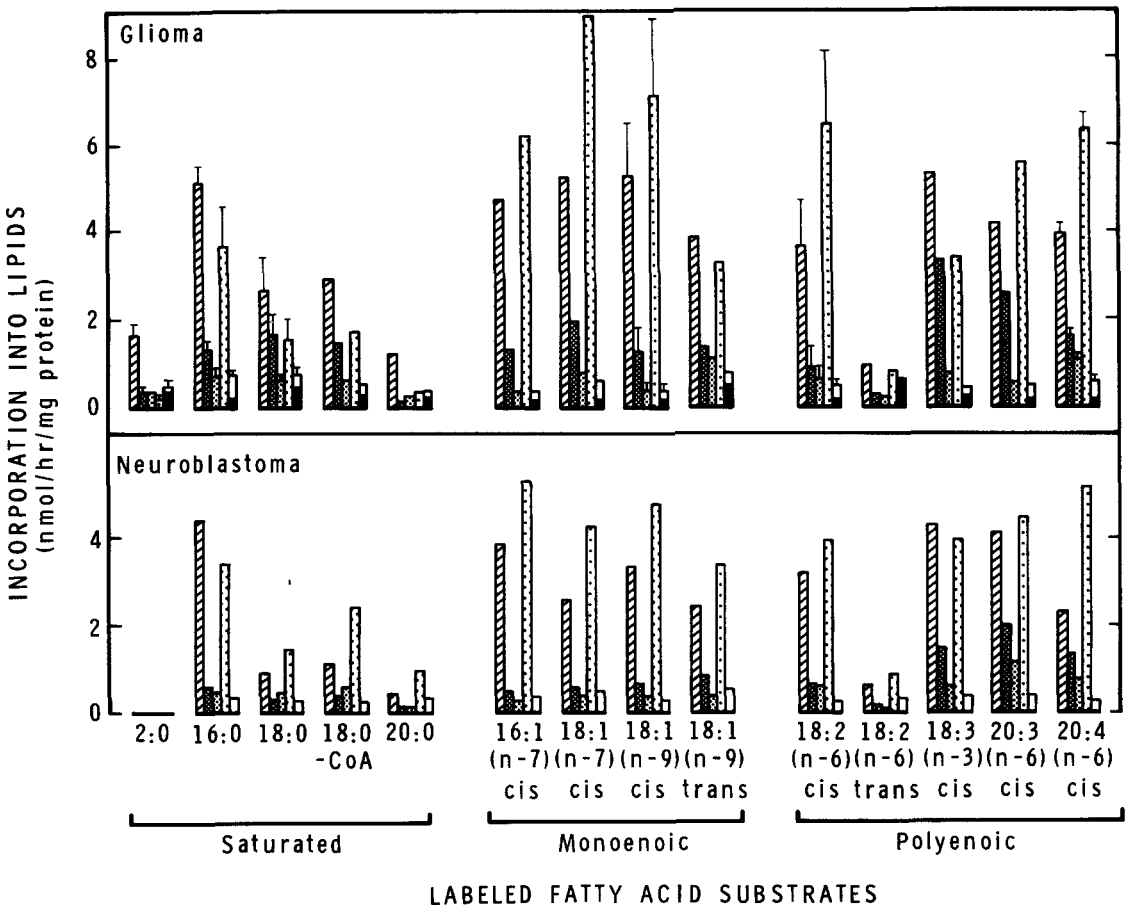

Fig. 2. Incorporation of fatty acids and precursors into neutral and phospholipid classes of glioma and neuroblastoma cells during 6-hr incubations. Conditions were as described for Fig. 1. ▨, choline phosphoglycerides; ▩, ethanolamine phosphoglycerides; ▩, inositol and serine phosphoglycerides; ▦, triacylglycerol; ▬, combined diacylglycerol and cholesterol fraction with cholesterol represented by the shaded portion of the bar (for glioma only).

incorporated at a lower rate than their *cis*-isomers. Saturated acid uptake by both cell lines was relatively variable and esterification varied inversely with chain length (16:0 > 18:0 > 20:0).

For all fatty acids, choline phosphoglycerides and triacylglycerol accounted for 60–85% of fatty acyl chain label (**Fig. 2**). With trienoic and tetraenoic substrates, ethanolamine, inositol, and serine phosphoglycerides also contained a large amount of labeled acyl chains.

Further analysis of the ethanolamine phosphoglyceride of neuroblastoma showed that nearly half (43–56%) of the label from the polyenoic acids was in plasmalogen; plasmalogen accounted for 53.1 ± 3.2% (n = 5) of total ethanolamine phosphoglyceride mass.

Products of fatty acid desaturation and chain elongation

Neuroblastoma. Acetate incorporation into neuroblastoma was insufficient to permit lipid class analysis. **Table 1** shows that exogenous palmitate and stearate incorporated into phospholipid had undergone desaturation, chain elongtion, or both. In triacylglycerol, the extent of desaturation and chain elongation was less. Unlike other saturated acids, exogenous arachidic acid underwent considerable β-oxidation before incorporation into phospholipid. Some arachidic was elongated to 22:0 and 24:0 before acylation. In marked contrast, label appearing in triacylglycerol was largely unchanged. Monoenoic acids (Table 1) were little metabolized except for palmitoleate, which was chain-elongated before incorporation into phospholipid. Elaidate was unique in that it appeared to undergo β-oxidation.

Considerable desaturation and/or elongation of *cis*-polyenoic substrates occurred before their incorporation into complex lipids (**Table 2**). At 6 hr, differences in incorporation of these products into phospholipid compared to triacylglycerol were relatively slight. An exception was arachidonic acid (20:4) which was more extensively chain-elongated prior to incorporation into triacylglycerol (33.2%) than into phospholipid (15.7%). The *trans*-18:2(n-6) isomer was handled differently than the corresponding *cis*-acid. There was some chain elon-

TABLE 1. Desaturation and chain elongation of saturated and monoenoic fatty acids by neuroblastoma and glioma cells in culture

		Phospholipid						Neutral Lipid					
		^{14}C-Substrate						^{14}C-Substrate					
Cell Line	Fatty Acid Formed	16:0	18:0	20:0	16:1 (n-7) *cis*	18:1 (n-9) *cis*	18:1 (n-9) *trans*	16:0	18:0	20:0	16:1 (n-7) *cis*	18:1 (n-9) *cis*	18:1 (n-9) *trans*
Neuroblastoma	16:0	67.3[a]		19.4			4.0	75.7	5.8	5.3[b]			4.2
	16:1	11.5			70.2			6.0			69.6		
	18:0	17.2	76.4	9.5				11.9	87.6	1.4			
	18:1	7.6	22.4	9.0	25.2	95.7	91.8	4.1	5.3	1.6	26.8	92.0	87.3
	20:0			44.9					4.4	79.8			
	20:1			2.7	4.8	4.3	3.2	0.2	0.3		3.3	6.8	8.4
	22:0			8.9					0.3	5.8			
	22:1			3.3						1.7			
Glioma	16:0	55.9	7.9	37.9		1.1	8.3	75.5	1.5			0.2	6.4
	16:1	19.4		9.0	63.1			8.9			53.5		
	18:0	9.2	45.9	9.2				6.8	88.9				
	18:1	15.6	46.3	14.4	30.8	95.4	91.3	8.5	9.5		40.8	87.6	83.3
	20:0			25.4						>90.0[c]			
	20:1			2.8		2.8						8.7	6.8
	22:0			6.3									
	24:0			2.4									

[a] Results are expressed as % of the radioactivity recovered in the total phospholipid or neutral lipid fraction following a 6-hr incubation with 100 μM substrate as indicated.

[b] For 20:0, the triacylglycerol fraction, isolated on TLC, is reported. A major proportion of total neutral lipid was free fatty acid, which was 99% 20:0.

[c] There was insufficient triacylglycerol for analysis. Free fatty acid accounted for about 90% of the total neutral lipid and was entirely unaltered 20:0. Thus, this is the minimum amount of unaltered 20:0 in the total neutral lipid fraction.

gation, but little or no desaturation; small amounts of label appeared in shorter chain acids, indicating β-oxidation.

Although polyenoic fatty acid patterns in phospholipids and triacylglycerol of neuroblastoma cells were similar at 6 hr (unlike the saturates, Table 1), there were major differences during the time course of incorporation. Examination of 18:2(n-6) at various incubation times (**Fig. 3**) indicated that >90% maximal uptake of fatty acid from the medium occurred during the first 6 hr, at which time <1% of the cell label was free fatty acid. Total label in triacylglycerol increased rapidly during the first 6 hr and declined thereafter, whereas label in total phospholipid increased 3-fold between 2 and 24 hr. Further, 20:4, a major end-product of 18:2(n-6) metabolism, accumulated primarily in phospholipid, whereas the intermediates 18:3, and to a lesser extent 20:3, were found in both phospholipid and triacylglycerol fractions.

The distribution of label from 18:2(n-6), 18:3(n-3),

TABLE 2. Products formed from polyenoic acids by neuroblastoma cells during 6-hr incubations

	Phospholipid					Neutral Lipid				
	^{14}C-Substrate					^{14}C-Substrate				
Fatty Acid Formed	18:2 (n-6) *cis*	18:2 (n-6) *trans*	18:3 (n-3) *cis*	20:3 (n-6) *cis*	20:4 (n-6) *cis*	18:2 (n-6) *cis*	18:2 (n-6) *trans*	18:3 (n-3) *cis*	20:3 (n-6) *cis*	20:4 (n-6) *cis*
18:2	89.1[a]	92.4				80.6	90.2			
18:3	3.0	1.8	77.0			5.8		78.7		
18:4			8.1					8.6		
20:2	6.0	1.2				5.8	7.1			
20:3	5.1	1.1	4.8	82.1		6.7	1.1	6.0	74.6	
20:4	4.8		10.2	10.3	84.3	1.8		6.6	19.4	68.8
22:3				3.4					3.6	
22:4				4.0	15.7				3.6	33.2

[a] Values are expressed as % of the radioactivity recovered in the total phospholipid and neutral lipid fractions of the substrates indicated.

20:3(n-6), and 20:4(n-6) in the major phospholipid fractions was analyzed. Although choline phosphoglycerides generally contained the largest quantity of altered and unaltered acyl chains, a major portion of the label in inositol phosphoglyceride was product formed by desaturation and/or chain elongation. Based on phospholipid concentrations, inositol phosphoglyceride had a much higher specific activity than other phospholipids with 20:3, 20:4, 20:5, and 22:4, either added as substrates or formed from other fatty acid precursors.

Glioma. Unlike neuroblastoma cells, cultured glioma cells incorporated significant amounts of labeled acetate appearing primarily as 16:0, 18:0, and 18:1 in both phospholipid and triacylglycerol. The longer chain saturated and monoenoic substrates showed patterns similar to those observed in neuroblastoma though the extent of metabolism was generally somewhat greater (Table 1). For polyenoic acids (data not shown), there was evidence of greater β-oxidation, especially of *trans*-18:2, and the extent of desaturation and chain elongation of 18:2(n-6) and 18:3(n-3) was greater than with neuroblastoma.

Product formation from the polyenoic fatty acids by glioma cells was also examined at different times of incubation; incorporation of 20:3(n-6) and 20:4(n-6) and their metabolic products into phospholipid and triacylglycerol is illustrated in **Fig. 4.** With these substrates (also with 18:2(n-6) or 18:3(n-3), data not shown) both

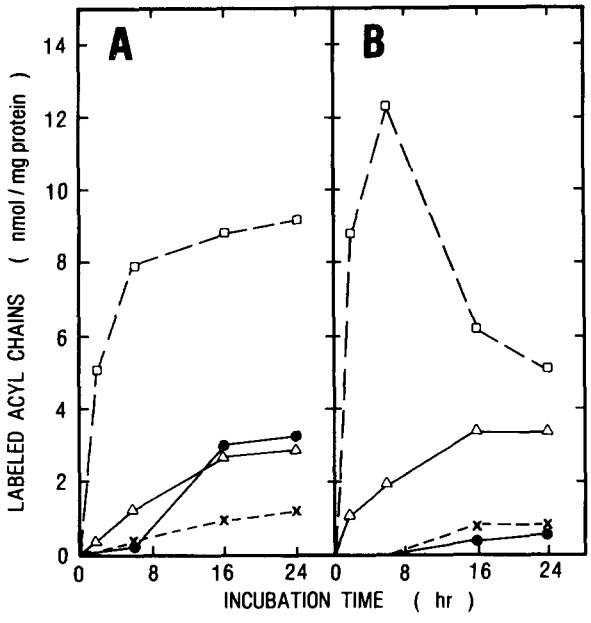

Fig. 3. Changes in the formation of products by neuroblastoma cells with time of incubation with [1-^{14}C]18:2(n-6). A, Phospholipid. B, Triacylglycerol. □ — — □, 18:2; △——△, 18:3; × — — ×, 20:3; ●——●, 20:4.

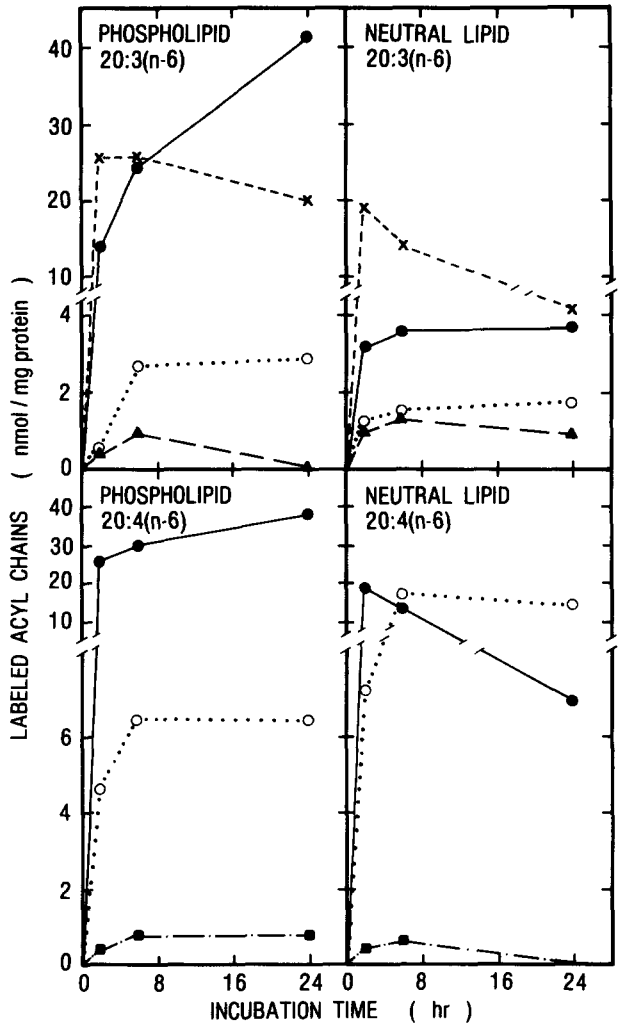

Fig. 4. Changes in the formation of product by C-6 glioma cells with time of incubation with [1-^{14}C]20:3(n-6) and [1-^{14}C]20:4(n-6). × — — ×, 20:3; ●——●, 20:4; ■ —·— ■, 20:5; ▲ — — ▲, 22:3; ○·····○, 22:4.

incorporation into phospholipid and metabolism by desaturation and chain elongation were more rapid than with neuroblastoma cells. As with neuroblastoma, total incorporation into triacylglycerol peaked and declined more rapidly than into phosholipid; also, 20:4(n-6) formed from 20:3(n-6) or added as a substrate accumulated to a greater extent in the phospholipids, giving a relative enrichment of the ethanolamine and inositol phosphoglyceride fractions. Products of direct chain elongation (22:3 from 20:3(n-6); 22:4 from 20:4(n-6); 20:2 from 18:2(n-6); 20:3 from 18:3(n-3)) accumulated to a greater extent and persisted longer in triacylglycerol. With 18:3(n-3) as substrate, the 20:5 formed was preferentially incorporated into phospholipids. For all substrates the proportion of minor intermediates declined between 8 and 24 hr.

DISCUSSION

Our experiments with neuroblastoma and glioma have extended previous observations to show that: *1*) all fatty acids of 16-carbons or greater were incorporated into complex lipids; and *2*) both cell lines were capable of extensive desaturation and chain elongation of acyl chains. In addition, we have demonstrated that: *3*) saturated fatty acids undergo β-oxidation and have a higher proportion in the free acid pool compared to unsaturated acids; *4*) *trans*-acids, particularly *trans* dienoic acid, are treated differently than their *cis*-isomers; and, *5*) triacylglycerol appears to act as a site of temporary storage for the influx of unsaturated fatty acyl chains.

Uptake, esterification to complex lipids, and metabolism of most long-chain fatty acids was very rapid. In contrast, acetate utilization from the medium was low with both cell types. Appreciable de novo synthesis from acetate did not occur in neuroblastoma cells, even in cells maintained 24 hr in media free of fatty acids prior to incubation, confirming observations by Volpe and Marasa (22, 23) that neuroblastoma cells preferentially utilize fatty acids from the medium and do not display active de novo synthesis from acetate.

In general, the distribution of labeled saturated and monoenoic fatty acyl chains in phospholipid classes was proportional to the phospholipid content of total cellular membrane resulting in similar specific activities of the major phospholipids. In contrast, for polyenoic acids, the specific activities of ethanolamine and inositol phosphoglycerides were greater than that for choline phosphoglyceride. Thus, selectivity in phospholipid synthesis ensures production of molecular species appropriate for cellular structure as demonstrated previously with various fractions of brain (24–30). Although we did not find as marked an enrichment in ethanolamine phosphoglycerides in 6-hr incubations with 18:3(n-3) as observed by Yavin, Yavin, and Menkes (5) with 24-hr incubations, the difference probably relates to greater conversion of 18:3(n-3) to 20:5 in their longer incubations. We did observe a much higher proportion of 20:3 and 20:4 formed from 18:3(n-3) in ethanolamine phosphoglyceride than for unaltered substrate. Also, about half of the ethanolamine phosphoglyceride radioactivity was associated with phosphatidyl ethanolamine confirming (3, 5) that plasmalogen is a major acceptor for polyunsaturated fatty acids of the (n-3) series.

For saturated acids, Δ9 desaturation and a single 2-carbon elongation predominated. Analyses of the products formed from substrates with two or more double bonds indicated that chain elongation both of the substrate and of products of Δ6 and Δ5 desaturation was an active process in both cell lines; Δ4 desaturation was not observed. From comparisons of neuroblastoma and primary cell cultures, Yavin et al. (5) suggested that neuroblastoma cells lose Δ4 desaturase activity during subcloning procedures, or that striking changes in the regulation of desaturation occur. Whether Δ4 desaturase activity is absent or merely suppressed under the culture conditions remains uncertain. Whatever regulates acyl chain unsaturation, neuroblastoma cells have a fatty acid profile that differs from normal neuronal cells, particularly in the lower levels of 20 and 22-carbon polyunsaturated fatty acids in membrane phospholipids (4).

In contrast to the unsaturated acids, large proportions of the saturated acids remained unesterified, particularly stearate (18:0) and arachidate (20:0). The relative proportion of free acid increased with increasing acyl chain length indicating relative exclusion of long-chain saturated fatty acids from esterification to membrane lipids. Arachidic acid appeared unique among the fatty acids tested, in that a major portion of label appeared in other chain fatty acids and cholesterol. The high proportions of labeled 16:0, 18:0, and 18:1 derived from 20:0 were unexpected in view of the low level of de novo synthesis from exogenous acetate or acetate derived from carboxyl-labeled 18 and 20-carbon polyenes. Possibly 20:0 stimulates β-oxidation and contributes more directly than exogenous acetate to the substrate pool for de novo synthesis. This may reflect a tendency for increasing β-oxidation as the chain length of saturated fatty acids increases. A less likely alternative is that retroconversion preserving the carboxyl label occurs.

Esterification of *trans*-acids was less than that of their *cis*-isomers. Both *trans*-acids tested were desaturated and elongated but no 20:4 was formed from *trans*-18:2, in agreement with earlier studies using intracerebral injections (12, 31). Label from both *trans*-acids appeared in palmitate and cholesterol, indicating that these substrates, like 20:0, were more susceptible to β-oxidation than other fatty acid substrates. Thus, these cultured cells possess selectivity on the basis of *trans*-double bonds, particularly with dienoic isomers. In general, our results confirm previous observations with brain (13) that the *trans*-monoenoic acid, elaidate, does participate to a limited extent in membrane-lipid synthesis.

For most fatty acids, triacylglycerol accounted for a large proportion of the esterified radioactivity after incubation up to 24 hr in the absence of serum. While products formed by desaturation and/or chain elongation were esterified to both triacylglycerol and phospholipids, there was greater incorporation of altered

acyl chains into phospholipids than in triacylglycerol. Thus, it seems that substrate acids and acyl chains modified by desaturation and elongation are not equally available for esterification to triacylglycerol. The tendency of exogenous fatty acids to label triacylglycerol and of endogenous products of desaturation and chain elongation to label phospholipids, along with a more rapid decrease of labeled triacylglycerol with longer incubations, support the possibility that exogenous unsaturated fatty acid substrates may be esterified to triacylglycerol initially, and then may be released for desaturation and chain elongation before preferential esterification to phospholipids. This possibility was originally suggested from previously reported studies of fatty acid metabolism in developing rat brain (32). While not an exclusive mechanism (some modified fatty acids are esterified to triacylglycerol and unaltered fatty acids to phospholipids), temporary storage of an influx of fatty acid might play a role in local supply or regulation of acyl chains during synthesis and turnover of membrane phospholipids.

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