Phospholipids and acyl groups of subcellular membrane fractions from human intracranial tumors

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Abstract The phospholipids from subcellular fractions of human intracranial tumors were examined. For comparison, microsomes were isolated from a fetal human brain and from the gray matter of adult human brains. The subcellular membranes of tumors had a higher protein-to-phospholipid ratio than the normal brain membranes. The microsomes from tumors had a lower proportion of diacylglycerophosphorylethanolamine and higher proportions of alkenylacylglycerophosphorylcholine and sphingomyelin (plus diacylglycerophosphorylinositol) than microsomes from the gray matter. Also, the ratios of alkenylacylglycerophosphorylethanolamine to diacylglycerophosphorylethanolamine were higher in the tumors than in the normal controls. The acyl groups of ethanolamine phosphoglycerides in tumor microsomes had relatively more 18:1, 18:2, and 20:4(n − 6) and less 18:0, 22:4(n − 6), and 22:6(n − 3) than the adult brain gray matter. Except for the increase in 18:2, acyl group changes in choline phosphoglycerides between tumors and controls were not as extensive as in the ethanolamine phosphoglycerides. The characteristic features of phospholipids and their constituent acyl groups of tumors were often present in all the subcellular fractions. Although the acyl group profiles of the tumor phosphoglycerides were in closer resemblance to the fetal brain than to the adult brain, other differences were observed. Results indicate that neoplastic brain cells are unique in their cellular composition, and consequently they deviate from the normal neurons and glials in metabolism and functions.

Supplementary key words microsomes · fetal brain · gray matter

An important aspect in the study of the cellular activity of neoplasms is an accurate account of the chemical constituents of their subcellular membranes. It is generally recognized that phospholipids are essential components of biological membranes, and the nonpolar side chains of the phospholipids often play an important role in the regulation of membrane functions. In order to understand the metabolic activity of neoplastic cells, it seems to be most pertinent to examine the phospholipids of their subcellular membranes. In an earlier study, Grossi, Paoletti, and Paoletti (1) demonstrated a gross difference in the fatty acid composition between human brain tumors and normal brain tissue. Subsequent studies (2–6) have also indicated alterations in some minor neutral lipid components of the brain tumors in general.

Although some attempts have been made to examine the phospholipids of brain tumors (7–9), interpretations of results have often been hindered by the cellular complexity of unfractionated tumor tissues taken for the analysis. Besides, there is little information concerning the acyl group composition of individual phospholipids in the membrane fractions (10). In a recent study, we reported that the distribution of phospholipids and acyl groups in subcellular membranes isolated from human brain is consistent with respect to age, sex, and diet (11). Furthermore, the lipid composition of microsomes isolated from human brain gray matter was different from that in white matter (11). In the present study, homogenates of human intracranial tumors were subjected to subcellular fractionation for the isolation of microsomes, mitochondria, and nuclei. The lipids from the microsomal fractions were examined and compared with other subcellular fractions of the tumors as well as the microsomes isolated from a fetal human brain and from adult human brains.

MATERIALS AND METHODS

Source of materials

Biopsy brain tumor samples were obtained within 30 min after surgical removal of the tumors. Tumor tissues were transferred to a sealed plastic container and were stored at −20°C until chemical analysis. The time period between surgery and chemical analysis usually did not ex-

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Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; diacyl-GPE, diacylglycerophosphorylethanolamine; diacyl-GPC, diacylglycerophosphorylcholine; diacyl-GPI, diacylglycerophosphorylinositol; diacyl-GPS, diacylglycerophosphorylserine.

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TABLE 1. Tumor diagnosis obtained by histological examination

Patient Data				
Tumor No.	Sex	Age	Tumor Diagnosis	Location in Brain
		yr		
1	Male	41	Astrocytoma grade I–II	Frontal lobe
2	Male	10	Astrocytoma grade III–IV	Cortex
3	Female	42	Glioblastoma	Right frontal lobe
4	Female	69	Glioblastoma multiforme	Right cortex
5	Female	65	Glioblastoma multiforme	Frontal lobe
6	Female	26	Meningioma	Right occipital cortex
7	Male	55	Hemangioblastoma	Cerebellum
8	Male	46	Malignant melanoma	Lymph node metastatic to cerebellum

Diagnosis of tumors was aided by examination of fixed and stained tumor tissue sections by light microscopy.

ceed 2 months. In some cases, brain tissues adjacent to the tumors were also collected for the analysis. The remaining portions of the tumors were taken for routine histopathological examinations. Diagnosis of tumors was aided by light microscopic examination of frozen and stained tissue sections (Department of Pathology, University of Oregon Medical School, Portland, Oreg.). **Table 1** gives a brief description of the diagnosis, the patient data, and the location of these tumors in the brain. For normal human brain tissues, portions of the frontal cortex were obtained from autopsies 4–10 hr after death of the subjects (11). The 20-wk-old fetal brain was obtained from a fetus after an abortion. Normal and fetal brain samples were treated similarly to the tumors.

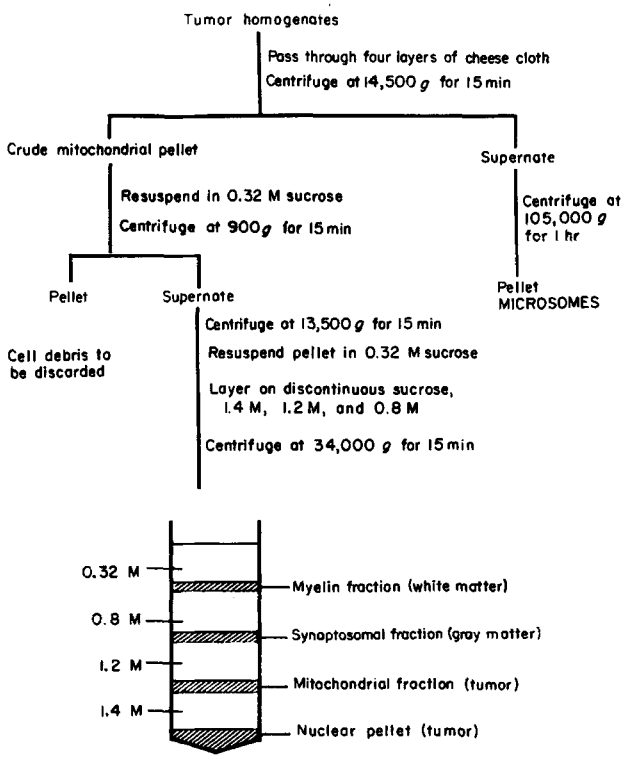

Fig. 1. The procedure for subcellular fractionation of brain tumor homogenates. Only microsomal, mitochondrial, and nuclear fractions were isolated for lipid analysis.

Subcellular fractionation

The procedures for separation of gray matter from white matter and for subcellular fractionation of normal brain tissues have been described (11). Prior to homogenization, brain and tumor tissues were rinsed several times with 0.32 M sucrose solution containing 1 mM EDTA and 15 mM Tris–HCl buffered at pH 7.4 in order to eliminate excessive blood contamination. Brain tissues were homogenized in 20 vol of the 0.32 M sucrose medium. The tumor homogenates were then filtered through eight layers of cheese cloth. We found that the procedure for subcellular fractionation of normal brain tissue (11, 12) was not suitable for the tumors; it was therefore modified as described in **Fig. 1.** In some experiments, the membrane pellets were fixed in glutaraldehyde and processed for examination of their ultrastructures by electron microscopy. The procedure for fixing and staining the pellets for electron microscopy has been described (13). The protein content of subcellular membranes was determined according to the method of Lowry et al. (14). After lipid extraction, the lipid phosphorus content of the same membrane fractions was determined according to the method of Gottfried (15).

Analysis of lipids

The final membrane pellets were each suspended in 7 ml of water, and total lipid was extracted with chloroform–methanol 2:1 (v/v) as described previously (11). For separation of the phospholipids, the total lipid extract was applied to thin-layer plates impregnated with silica gel G and Na_2CO_3 (the silica gel G slurry was made up in 0.01 M Na_2CO_3). Phospholipids were separated according to the separation–reaction–separation procedure described by Horrocks and Sun (16) but with the solvent system described by Sun and Sun (17). After development, the lipids on the plates were visualized by exposing the plates to iodine vapor. Individual phospholipid spots were scraped from the TLC plate into test tubes for determination of the lipid phosphorus content (15). In some TLC separations, sphingomyelin and diacyl-GPI were not clearly resolved, and these areas were therefore combined for lipid phosphorus assay. In some instances when these two lipid spots were clearly separated, we noticed that the amount of sphingomyelin in tumors was generally higher than the diacyl-GPI.

For analysis of acyl groups, the phospholipids were separated by two-dimensional TLC without exposing the plates to HCl fumes (16). After the plates were developed, lipids were visualized by spraying the thin-layer plates with 2% 2'7'-dichlorofluorescein in ethanol. The acyl groups of individual phosphoglycerides were converted to their methyl esters by alkaline methanolysis (18). The methyl esters were then analyzed by GLC as described previously (12).

RESULTS

Subcellular fractionation of human brain tumors

After centrifugation of the tumor homogenates at 14,500 *g* for 15 min, the sedimented pellets were generally grayish with small reddish brown portions at the bottom. These pellets were resuspended in 0.32 M sucrose, and further centrifugation yielded mainly the nuclear fraction **(Table 2).** When normal brain homogenates were subjected to subcellular fractionation, the major fractions were myelin and synaptosomes depending on the proportion of gray and white matter present. The microsomal pellets isolated from the tumors were reddish. Based on electron microscopic examination, the microsomes isolated from brain tumors were quite homogeneous and were apparently not contaminated by intact red blood cell membranes or collagen **(Fig. 2).** Although a few small mitochondria may have been present in this fraction, contamination by myelin and by synaptosomal particles was quite rare. The microsomal membranes from the tumors were vesicular in nature and resembled the rough endoplasmic reticulum with ribosomes still attached to the membranes. Electron microscopic examination of the mitochondrial–synaptosomal fraction from the tumor homogenates indicated that the membranes were quite heterogeneous. Unlike those isolated from the normal brain homogenates, there were very few synaptosomes present, and those that were present appeared to be greatly "degenerated," with no defined synaptic components. Occasionally, some glycogen granules were also present. Due to the heterogeneity and the low yield, a detailed biochemical analysis of the mitochondrial fractions was not performed.

Phospholipid composition of brain tumors

As shown in Table 2, the protein-to-phospholipid ratios of cellular membranes were higher in tumors than in the brain homogenates. The phospholipids from human gray matter microsomes contained a high proportion of diacyl-GPC and a relatively low proportion of sphingomyelin **(Table 3).** The ratios of alkenylacyl-GPE to diacyl-GPE in the microsomes from gray matter were less than 1. The proportion of sphingomyelin (plus diacyl-GPI) in the one astrocytoma analyzed was almost three times higher than that in the gray matter. The two glioblastomas also had elevated proportions of sphingomyelin (plus diacyl-GPI), although to a different extent. In general, the tumors had lower proportions of diacyl-GPE, and the ratios of alkenylacyl-GPE to diacyl-GPE were greater than 1. Ordinarily, alkenylacyl-GPC was not detected in normal brain tissue, but a significant amount of this phospholipid was found in the tumors, especially in the two glioblastomas analyzed.

The phospholipid compositions of the mitochondrial fraction from an astrocytoma and the nuclear fraction from a glioblastoma were compared with the corresponding microsomal fractions **(Fig. 3).** Both microsomes and mitochondria of the astrocytoma had a high content of sphingomyelin in the subcellular fractions. There were high proportions of alkenylacyl-GPE found in the microsomes of the glioblastoma as well as in the nuclear membranes.

TABLE 2. Percentage distribution of protein and the ratio of protein to phospholipid among subcellular membranes isolated from human intracranial tumors

Tumor	Myelin		Microsome		Synaptosome and/or Mitochondria		Nuclear	
	%	*ratio*	%	*ratio*	%	*ratio*	%	*ratio*
Astrocytoma (1)[a]			21	2.5	14	3.8	66	6.4
Glioblastoma (3)			53	3.2	12	2.3	35	9.1
Tissue adjacent to tumor (3)	15		12	2.1	11	3.8	63	6.7
Glioblastoma (4)			42	2.8	6	1.2	52	6.1
Tissue adjacent to tumor (4)	36		43	1.6	10		12	4.5
Normal brain tissue[b]	58	1.1	24	1.0	15	1.7	2	4.1

The procedure for subcellular fractionation is described in text. After isolation, membrane pellets were suspended in water and portions were taken for determination of protein and phospholipid content.

[a] Numbers in parentheses refer to the tumor numbers in Table 1.

[b] Biopsy brain sample containing both gray and white matter was obtained from a patient during frontal lobe craniotomy. This patient had no neurological abnormality.

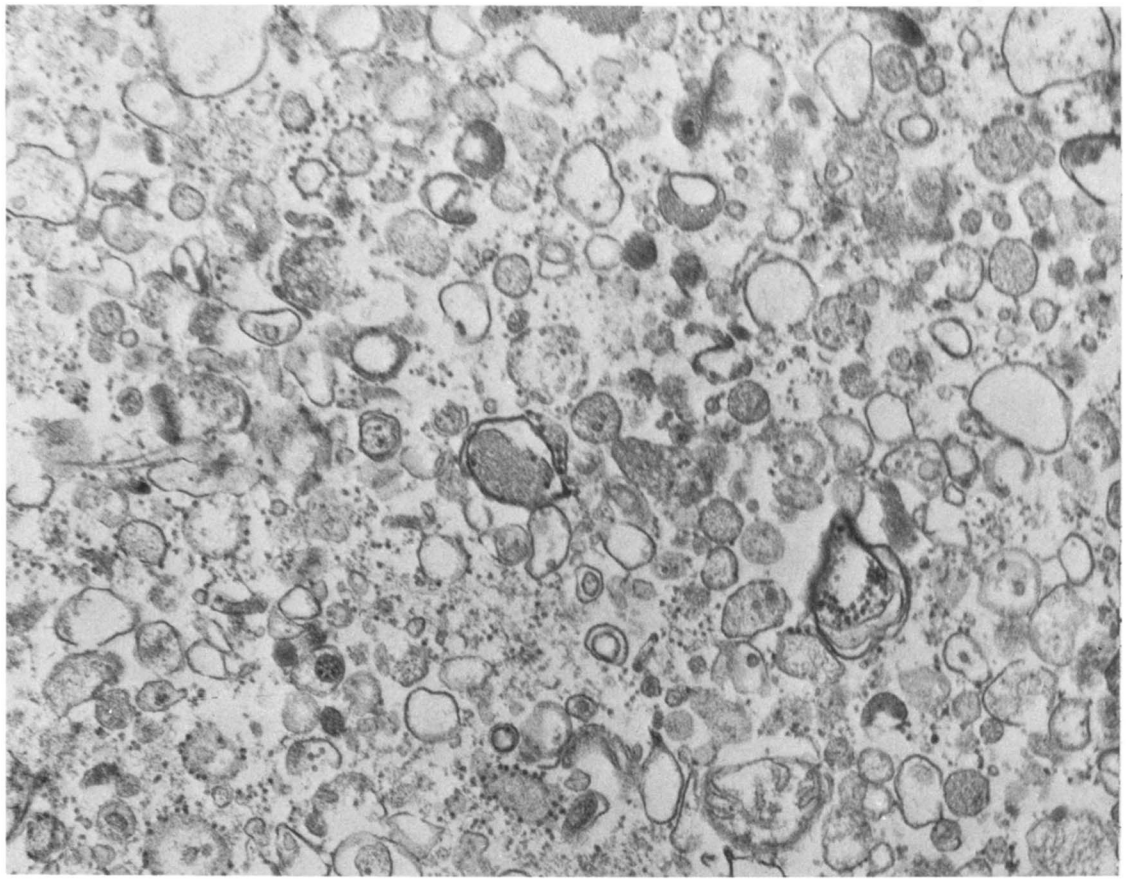

Fig. 2. An electron micrograph of the microsomal membranes isolated from a glioblastoma. Magnification ×30,000.

Acyl group composition of tumor phosphoglycerides

Due to the small amount of tissue available for subcellular fractionation, preliminary acyl group analysis was limited primarily to the ethanolamine and choline phosphoglycerides. Besides the astrocytomas and the glioblastomas, we have included in the acyl group analysis three other intracranial tumors of nonneural cellular origin (**Tables 4** and **5**). However, for comparison with micro-

TABLE 3. Composition of phospholipids of microsomes in intracranial tumors and cortical gray matter

Tumor	Diacyl-GPS	Sphingomyelin + Diacyl-GPI	Alkenyl-acyl-GPC	Diacyl-GPC	Alkenyl-acyl-GPE	Diacyl-GPE	Other
				% lipid phosphorus			
Astrocytoma (1)[a]	11.3	29.4	0.5	34.5	14.3	10.1	
Glioblastoma (3)	10.4	22.0	2.8	37.7	15.7	9.9	1.4
Tissue adjacent tumor (3)	13.8	13.5		48.7	12.9	11.1	
Glioblastoma (4)	10.9	13.8	3.1	36.0	24.7	11.6	
Cortical gray matter[b]	11.3	10.6[c]		37.4	17.3	21.1	1.8

Lipids from the microsomal pellets were extracted with chloroform–methanol 2:1 (v/v). Phospholipids were separated by two-dimensional TLC as described in the text. Individual lipid areas were scraped from a TLC plate for the determination of phosphorus content. Results are expressed as mole % of the total lipid phosphorus content. Abbreviations: GPS, glycerophosphorylserine; GPI, glycerophosphorylinositol; GPC, glycerophosphorylcholine; GPE, glycerophosphorylethanolamine.

[a] Numbers in parentheses refer to tumor numbers in Table 1.

[b] Microsomes from cortical gray matter were results of 10 analyses. Subject age ranged from 61 to 87 yr. See Ref. 11 for SEM.

[c] The mean value for sphingomyelin was 5.0, and that for diacyl-GPI was 5.6.

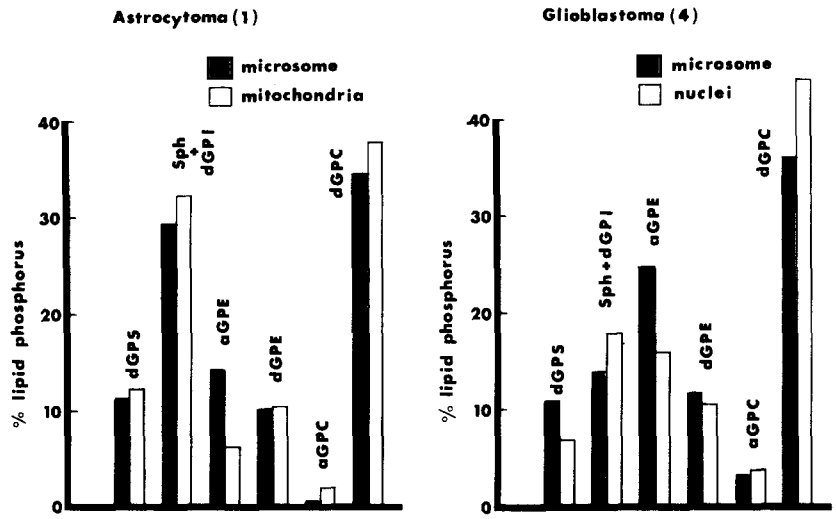

Fig. 3. Phospholipid composition of subcellular fractions isolated from astrocytoma (tumor 1) and glioblastoma (tumor 4). Phospholipids were separated by reactional two-dimensional TLC as described in the text. Values are expressed as percentages of total lipid phosphorus content. Abbreviations: dGPS, diacylglycerophosphorylserine; Sph, sphingomyelin; dGPI, diacylglycerophosphorylinositol; aGPE, alkenylacylglycerophosphorylethanolamine; dGPE, diacylglycerophosphorylethanolamine; aGPC, alkenylacylglycerophosphorylcholine; dGPC, diacylglycerophosphorylcholine.

somal lipids from normal brain and fetal brain, only the astrocytomas and glioblastomas were grouped (**Tables 6 and 7**) for statistical treatment. Data from astrocytoma (tumor 1) were not included because the acyl group and phospholipid profiles were peculiar and were distinctly different from those of the other tumors. Compared with the microsomes from normal brain gray matter (Table 6), the acyl groups of ethanolamine phosphoglycerides in the tumors had lower proportions of 18:0, 22:4(n − 6), and 22:6(n − 3) and higher proportions of 18:1, 18:2, and 20:4(n − 6). In addition, there was slightly more of the minor acyl groups such as 20:3(n − 6) and 22:5(n − 3) in the tumors than in the normal brain tissues.

In general, the acyl group profile of choline phosphoglycerides was different from that of the ethanolamine phosphoglycerides, regardless of whether they were from tumor or normal brain tissues (Tables 5 and 7). The choline phosphoglycerides were rich in 16:0, 18:0, and 18:1 but contained relatively small amounts of the long-chain polyunsaturated fatty acids. Except for the increase in 18:2 and 20:4(n − 6), acyl group changes in choline phosphoglycerides in the tumors were not as extensive as in the ethanolamine phosphoglycerides.

TABLE 4. Acyl group composition of ethanolamine phosphoglycerides in the microsomal fraction from human intracranial tumors

Fatty Acid	Astrocytomas		Glioblastomas					Meningioma	Hemangio-blastoma	Melanoma
	(1)[a]	(2)	(3)	(3A)	(4)	(4A)	(5)	(6)	(7)	(8)
					% by weight					
16:0[b]	6.6	7.5	7.5	8.0	4.3	6.3	7.6	12.6	7.3	7.3
16:1									2.0	2.0
18:0	5.1	11.7	11.3	14.6	8.3	15.3	14.0	17.0	13.6	12.4
18:1	45.3	15.0	24.7	22.3	17.1	15.4	16.9	19.7	18.6	32.0
18:2	1.4	3.0	2.1	1.7	3.2	4.0	4.9	3.6	6.5	11.8
18:3	1.0		1.5	2.4			6.2	1.2	3.8	1.9
20:1	1.1	0.9		0.6	0.7	1.0	0.7	0.5	0.5	
20:3(n − 6)	0.6	1.2	1.6	1.5	1.3	1.2	1.3	1.4	1.9	0.8
20:4(n − 6)	21.3	37.7	30.9	21.7	39.9	25.4	21.8	30.0	26.1	20.4
22:4(n − 6)	3.9	13.5	6.9	8.9	7.7	10.6	13.1	4.5	7.6	2.3
22:5(n − 6)	1.2	1.0	0.9	0.5	3.6	3.0	1.3	1.5	1.2	
22:5(n − 3)		3.0	0.9		1.5	0.6			3.3	1.9
22:6(n − 3)	7.9	5.5	10.9	13.9	12.5	16.6	12.2	7.9	9.7	6.6

Lipids were separated by two-dimensional TLC as described in the text. The acyl groups from the phospholipids were converted to fatty acid methyl esters and subsequently analyzed by GLC.

[a] Numbers in parentheses refer to tumor numbers in Table 1. "A" denotes brain tissue adjacent to tumors.

[b] Number of carbon atoms: number of double bonds.

TABLE 5. Acyl group composition of choline phosphoglycerides in the microsomal fraction from human intracranial tumors

Fatty Acid	Astrocytomas		Glioblastomas					Meningioma	Hemangio-blastoma	Melanoma
	(1)[a]	(2)	(3)	(3A)	(4)	(4A)	(5)	(6)	(7)	(8)
					% by weight					
14:0[b]	0.9		1.6	0.9	2.0			0.9		3.4
16:0	46.7	40.3	42.8	51.0	43.9	46.9	39.2	42.1	35.0	37.8
16:1	8.9		6.8	1.4	1.0		0.7	0.7		3.2
18:0	1.8	9.0	4.4	6.2	6.9	7.3	10.3	8.8	10.4	8.9
18:1	37.6	28.7	33.4	32.8	29.6	31.5	25.0	26.4	30.2	26.3
18:2	0.6	3.2	2.2	1.8	4.6	3.5	5.1	3.8	12.4	14.7
18:3		2.4		0.8		0.9	5.2	2.1	1.7	
20:1					0.5	0.6				0.8
20:3(n − 6)		1.2	1.0		1.2	0.8	1.0	2.1	1.8	
20:4(n − 6)	2.6	9.7	6.1	4.4	7.1	5.7	6.5	10.4	6.5	3.7
22:4(n − 6)		3.0	0.7		1.0	1.2	3.8	1.2	0.3	
22:6(n − 3)		1.1			1.2	1.4	3.2	1.6	1.3	

Lipids were separated by two-dimensional TLC as described in the text. The acyl groups from the phospholipids were converted to fatty acid methyl esters and subsequently analyzed by GLC.

[a] Numbers in parentheses refer to tumor numbers in Table 1. "A" denotes brain tissue adjacent to tumors.

[b] Number of carbon atoms: number of double bonds.

The acyl group composition of phosphoglycerides from the meningioma (tumor 6) was similar to the astrocytomas and glioblastomas indicated in Tables 6 and 7. However, the acyl group compositions of the hemangioblastoma (tumor 7) and melanoma (tumor 8), which were located in the cerebellum, were different and seemed to have a higher level of 18:2 in both the ethanolamine and choline phosphoglycerides.

The acyl groups of ethanolamine phosphoglycerides in the fetal brain had a higher proportion of 20:4(n − 6) and 22:4(n − 6) and a lower proportion of 22:6(n − 3) than the normal gray matter (Table 6). In the choline phosphoglycerides (Table 7), an obvious difference was a decrease in the proportion of 16:0 in the fetal brain.

The acyl group composition of ethanolamine phosphoglycerides from the microsomal fraction of glioblastoma (tumor 4) was compared to that of the mitochondrial and nuclear fractions **(Fig. 4)**. This glioblastoma had a high proportion of 20:4(n − 6), and the results indicated that this characteristic feature was manifested in the major subcellular fractions analyzed.

DISCUSSION

Results from the present study have demonstrated the differences in phospholipids and their acyl group profiles of subcellular membranes from intracranial tumors and

TABLE 6. Acyl group composition of ethanolamine phosphoglycerides in the microsomes from human brain gray matter, fetal brain, and tumors

Fatty Acid	Tumors[a]	Fetal Brain (20 wk old)	Gray matter	
			14–56 yr[b]	61–84 yr[b]
		% by weight		
16:0	6.7 ± 0.81	2.0	5.2 ± 0.24	4.6 ± 0.43
18:0	11.3 ± 1.17	21.3	24.8 ± 0.36	24.6 ± 0.66
18:1	18.4 ± 2.15	8.4	11.8 ± 0.88	11.6 ± 0.64
18:2	3.3 ± 0.59		0.7 ± 0.12	0.7 ± 0.15
18:3	1.9 ± 1.47	0.8		
20:1	0.6 ± 0.20	0.6	0.8 ± 0.08	0.8 ± 0.13
20:3(n − 6)	1.4 ± 0.09	0.5	0.6 ± 0.15	0.7 ± 0.06
20:4(n − 6)	32.6 ± 4.07	21.5	14.3 ± 0.64	13.0 ± 0.26
22:4(n − 6)	10.3 ± 1.74	17.0	12.7 ± 0.30	12.2 ± 0.49
22:5(n − 6)	1.7 ± 0.64	7.2	1.7 ± 0.32	1.2 ± 0.22
22:5(n − 3)	1.4 ± 0.63			
22:6(n − 3)	10.3 ± 1.63	17.0	27.1 ± 1.32	31.1 ± 1.11
	(4)	(1)	(5)	(7)

Numbers in parentheses indicate number of individual analyses.

[a] Values are means ± SEM from astrocytoma (tumor 2) and glioblastomas (tumors 3, 4, and 5).

[b] Range in age of subjects.

TABLE 7. Acyl group composition of choline phosphoglycerides in microsomes from human brain gray matter, fetal brain, and tumors

Fatty Acid	Tumors[a]	Fetal Brain (20 wk old)	Gray Matter 14–56 yr[b]	Gray Matter 61–84 yr[b]
		% by weight		
14:0	0.9 ± 0.53			
16:0	41.6 ± 1.09	34.3	49.3 ± 0.52	46.6 ± 2.05
16:1	2.1 ± 1.57	1.6		
18:0	7.7 ± 1.29	13.5	10.5 ± 0.45	11.7 ± 0.66
18:1	29.2 ± 1.73	35.5	30.1 ± 0.61	30.8 ± 0.67
18:2	3.8 ± 0.66		1.0 ± 0.35	0.8 ± 0.17
18:3	1.9 ± 1.24	1.8		
20:1		1.0	0.8 ± 0.24	0.7 ± 0.08
20:3(n − 6)	1.1 ± 0.06		0.5 ± 0.15	0.5 ± 0.06
20:4(n − 6)	7.4 ± 0.81	7.0	4.2 ± 0.31	4.2 ± 0.15
22:4(n − 6)	2.1 ± 0.76	1.5	0.9 ± 0.08	1.3 ± 0.15
22:6(n − 3)	1.4 ± 0.67	1.6	2.2 ± 0.12	2.9 ± 0.45
	(4)	(1)	(5)	(7)

Numbers in parentheses indicate number of individual analyses.
[a] Values are means ± SEM from astrocytoma (tumor 2) and glioblastomas (tumors 3, 4, and 5).
[b] Range in age of subjects.

human brain gray matter. Histological examination has shown that tumor cells contain large, pleomorphic nuclei and are often multinucleated (9). These nuclei formed the major subcellular fraction of the tumor homogenates. On the other hand, the subcellular fractions from nontumor brain tissues yielded mainly synaptosomes and myelin, depending on the proportion of gray or white matter present (Table 2). The lack of synaptic components, together with the near absence of (Na^+, K^+)-ATPase activity in the tumor homogenates,[2] strongly suggests that the neoplastic cells in brain do not have the functional activities of the neurons and glials.

In an earlier study, we reported the differences in lipid composition of microsomes isolated from the gray matter and the white matter (11). These results seem to indicate that specificity in lipid synthesis may be governed by the cell, which in turn is related to the type of membranes in the cells. The lipid compositions of subcellular membranes from the two major cell types in the gray matter are similar (19, 20) but are different from that of the oligodendroglia from the white matter (21). Since the astrocytomas and glioblastomas are of neuronal origin, we have isolated microsomes from the cortical gray matter for comparison.

The distribution of phospholipid in microsomes from the gray matter is similar to that of the synaptosomes (11) and gray matter homogenates reported previously by Svennerholm (22). The distribution of phospholipids in tumors, however, seems to vary widely. This heterogeneity in phospholipid distribution in the tumors has been noted by other investigators (7–10) using total homogenates for the analysis. We have further observed that the characteristic features in the microsomes are also manifested in other subcellular membrane fractions (Fig. 2). The heterogeneous nature of the tumor phospholipids is therefore not due to contamination but is more likely related to the degree of cellular differentiation and the metastatic property of individual tumors. In general, the tumor lipids have a higher proportion of sphingomyelin (plus diacyl-GPI) and a lower proportion of diacyl-GPE than microsomes from gray matter. In the few tumors analyzed here, we have not observed the drastic increase in the proportion of diacyl-GPC reported by others (7). Although the amount of alkenylacyl-GPE present in tumors may be less than that in normal brain due to the overall decrease in phospholipids, the ratio of alkenylacyl-GPE to diacyl-GPE in the tumors is generally higher than that in the microsomes from gray matter. Since choline plasmalogens

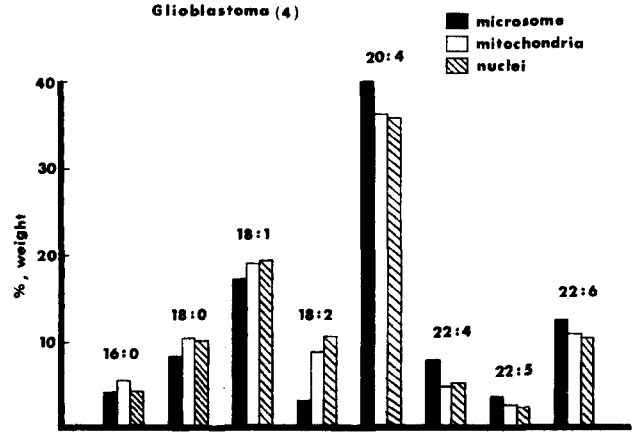

Fig. 4. The acyl group composition of ethanolamine phosphoglycerides in the microsomal, mitochondrial, and nuclear fractions from glioblastoma (tumor 4).

[2] Sun, G. Y. Unpublished data.

are not normally detected in brain, their presence in the tumors together with the increase in ethanolamine plasmalogens with respect to the diacyl-GPE suggest that the metabolism of plasmalogens during neoplastic transformation is abnormal. The increase in plasmalogens in the phospholipids seems to be in agreement with the general increase in alkyl and alkenyl ethers of glycerol in neoplastic human tissues (2).

The acyl group composition of phosphoglycerides in the microsomal fraction of the brain tumors is in general similar to that reported by White (10) for the tumor homogenates. An obvious feature of the acyl groups of phosphoglycerides in tumors is the increase in 18:2, which is found in both ethanolamine and choline phosphoglycerides. Since 18:2 is abundant in the phospholipids of serum and red blood cell membranes but not in normal brain tissues, its association with the brain tumors implies that the neoplastic brain cells may have lost the specificity in fatty acid uptake. It is also possible that the transformed cells, being in a stage of rapid proliferation, are utilizing nutrients from the blood due to vascular infiltration.

The neuronal membranes in brain are known to contain a high proportion of 22:6(n − 3) (17–23), whose function is believed to be the maintenance of the membranes in a semifluid state in order to facilitate the translocation of molecules. Since neoplastic brain cells are not designed to carry out normal neuronal functions, a high level of 22:6(n − 3) in their membranes would then be unnecessary. In the fetal brain, where cells are also in a rapid growing stage, the acyl groups of ethanolamine phosphoglycerides showed a higher proportion of 20:4(n − 6) and 22:4(n − 6) and a lower proportion of 22:6(n − 3) than the mature brain. Our results with respect to acyl group composition of fetal and adult brain are in good agreement with those reported previously by Svennerholm (22). Like the fetal brain membranes, these brain tumors also had relatively more 20:4(n − 6) and less 22:6(n − 3) in the phosphoglycerides compared with the adult human brain. Possibly, a higher proportion of 20:4(n − 6) is characteristic of rapid cell growth, whereas the increase in 22:6(n − 3) during development may be an indication of the maturity of the synaptic membranes. The acyl group changes towards that of the fetal brain composition also suggest that neoplastic cells are less differentiated than the adult neurons. In spite of the observed similarities, tumors are different from the fetal brain lipids in other ways, such as the differences in proportions of 18:1 and 22:4(n − 6). In the fetal brain, the increase of 20:4(n − 6) in ethanolamine phosphoglycerides is also accompanied by the increase of 22:4(n − 6), an elongated product of 20:4(n − 6) (Table 6). However, this is not seen in the tumors.

The acyl groups of ethanolamine phosphoglycerides from the tumors had a higher proportion of unsaturated fatty acids than the adult brain gray matter (Table 6). However, a more interesting finding is the marked increase in 20:4(n − 6) and decrease in 22:6(n − 3) fatty acids in the tumors. Since the increase in 20:4(n − 6) is not accompanied by an increase in 22:4(n − 6), the results suggest that the neoplastic brain cells may lack the enzymes for the elongation and desaturation processes. Results have demonstrated obvious alterations in membrane lipid composition of neoplastic brain cells. Consequently, further studies are necessary for correlating the altered membrane lipids with cellular metabolism and functions during transformation and malignancy.

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