

Lipid composition of human neural tumors

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Abstract Gangliosides, cholesterol, and phospholipids were quantitated in the tissues of 11 human neural tumors and the cells of two gliomas cultured in vitro. All tumor tissues contained higher water concentrations but lower total lipid concentrations than either human grey or white matter. In general they contained less cholesterol, sphingomyelin, and serine glycerophospholipid but more choline glycerophospholipid than white matter. Concentrations of total ganglioside sialic acid were intermediate between grey and white matter. Compared with normal brain, all tumors had greater proportions of the structurally less complex gangliosides and smaller proportions of the more complex gangliosides. This was most marked in the rapidly growing tumors while the better differentiated astrocytomas contained the greatest proportions of complex gangliosides. The cells of the cultured tumors contained amounts of total lipid and total phospholipid similar to their parent tissues. However, the cultures had less cholesterol, sphingomyelin, and total ganglioside than their parent tissues. There were significant amounts of choline and ethanolamine plasmalogen in both cultures and parent tissues. The ganglioside patterns of both cultures were complex but they contained a greater proportion of structurally simpler gangliosides than their parent tissues.—Yates, A. J., D. K. Thompson, C. P. Boesel, C. Albrightson, and R. W. Hart. Lipid composition of human neural tumors. *J. Lipid Res.* 1979. **20**: 428–436.

Supplementary key words ganglioside · phospholipid · plasmalogen · astrocytoma · glioma · neuroblastoma · tissue culture · tumor

Malignant tissues are characterized by the uncontrolled proliferation of their cells. Evidence suggests that cell surface components may participate in regulation of cell growth (1–3), and many types of tumor cells have been reported to have ultrastructural abnormalities of their cell surfaces (4). Changes in the composition of glycosphingolipids, cell surface components which may be involved in intercellular adhesion and regulation of cell growth (5), often accompany the malignant transformation of cells (6–8). For example, changes in the composition and metabolism of gangliosides, a subclass of glycosphingolipids, have been found to accompany viral transformation of cells (9). In man, Kostic and Buccheit (10) found abnormal ganglioside patterns in some gliomas that

may correlate with the degree of glioma malignancy. In the present studies we have analyzed the ganglioside composition of several different histological types of human primary neural tumors. Furthermore, we have cultured two human gliomas in sufficient quantities to determine their contents of gangliosides as well as of cholesterol and phospholipids.

METHODS

Tumor specimens

Neoplastic neural tissues obtained at the time of surgery were dissected by a neuropathologist (either C.P.B. or A.J.Y.) into portions for diagnostic light microscopy and chemical analyses. Those for microscopic examination were placed in 10% formalin; those for chemical analyses were frozen in normal saline at -40°C . Considerable care was taken to avoid necrotic or hemorrhagic tissues and to chemically analyze tissues of gross appearance and location as similar as possible to those used for microscopic examination.

Formalin-fixed tissues were processed for light microscopy and stained with both the hematoxylin and eosin and the phosphotungstic acid–hematoxylin methods. In several cases portions fixed in Cajal's fixative were stained with Hortega's silver carbonate stains (for connective tissue, and triple impregnation). Tumors were classified using the criteria and nosology of Russell and Rubinstein (11).

Tissue culture

One astrocytoma and one glioblastoma were cultured in sufficient quantities for chemical studies (Table 4). Tumor specimens removed at the time of craniotomy were received and transported under sterile conditions to the tissue culture laboratory. Portions

Abbreviations: FCS, fetal calf serum; IGP, inositol glycerophospholipid; SGP, serine glycerophospholipid; CGP, choline glycerophospholipid; EGP, ethanolamine glycerophospholipid; Sph, sphingomyelin; Plas, plasmalogen.

of the tumor were aseptically dissected and minced by the cross blade method before being placed into suspension by vigorous pipetting in Modified Earle's Medium-NCTC-135 (GIBCO) containing 10% fetal calf serum (FCS) (Flow Laboratories, Rockville, MD.) Aliquots of this suspension were placed in primary culture in Corning 75 cm² tissue culture flasks. The cultures were maintained at 37°C in a 5% CO₂ humidified environment. They were examined by means of phase contrast microscopy and, when growth was confluent, the cells were harvested after trypsinization (12). Aliquots of trypsinized cells were counted in a hemocytometer and the remainder were passed. To decrease fibroblast contamination, cultures were grown in media containing 20% gamma-globulin-free FCS (GIBCO) for 12 hr (13). The medium was then changed to Modified Earle's Medium-NCTC-135 with 10% FCS. Cells were fed with this medium three times per week until they had grown to confluency. Samples between passages 5 and 15 for chemical analyses were harvested at confluency with a rubber policeman, washed with phosphate-buffered saline, centrifuged in a Sorvall centrifuge at 800 rpm for 10 min, and the pellets were pooled and frozen at -40°C. Cultures were examined frequently by phase contrast microscopy but samples to be stained for light microscopy were grown on glass coverslips, fixed in absolute methanol, and stained with the Jacobi stain (14).

Chemical analyses

Immediately after thawing, the tissues were blotted dry and weighed. Lipids from both tumor tissues and cell cultures were extracted and partitioned by the method of Suzuki (15) and the lower phase was washed three additional times with theoretical upper phase containing water. Gangliosides were purified as described by MacMillan and Wherrett (16) and ganglioside sialic acid was estimated by the method of Svennerholm (17) as modified by Miettinen and Takki-Luukkainen (18). Aliquots of purified ganglioside containing 15 µg of sialic acid were separated by thin-layer chromatography in duplicate. The solvent system chloroform-methanol-water-concentrated ammonium hydroxide 60:35:7:1 (by volume) was used to develop 0.5-mm thick silica gel G (Analtech) plates; hard precoated silica gel plates purchased from Merck were developed with chloroform-methanol-0.02% CaCl₂ 60:40:10. Only the gangliosides from the two tumors that were cultured and the cultured cells derived from them were separated by the latter method, which separates GD1a and GD3 better than the former. Gangliosides separated by either method were quantitated using the method of MacMillan and Wherrett (16). Phospholipids were separated on

Analtech thin-layer plates 20 × 20 cm coated with 0.5-mm silica gel G following the technique of Rouser, Kritchevsky, and Yamamoto (19). Plasmalogens of the tissue culture specimens and their parent tissues were separated as described by Horrocks and Sun (20). Cholesterol contents of washed lower phase lipids were estimated as described by Zak and Epstein (21) and protein content of nonlipid residue was determined by the method of Lowry et al. (22). The amounts of total lipid and nonlipid residue were determined gravimetrically (19), and water was determined by difference between fresh weight and total dry weight.

RESULTS AND DISCUSSION

Eleven tumors were studied. Four were primitive neuroectodermal tumors of which two were peripheral neuroblastomas, one a cerebellar medulloblastoma, and one a cerebral primitive neuroectodermal tumor. These have been considered as one group because they are all primitive neural tumors. The other seven tumors were astrocytic in nature; of these two were differentiated, two were anaplastic, and three were glioblastomas multiforme.

The water contents of all the tumors tested in our laboratory were higher than those of either white matter or grey matter of normal human brain (Table 1). The high water content of neural tumors has been noted before and is higher than that which has been found in many intracranial tumors of non-neural origin (24, 25). This high water content may be due partially to edema associated with a deficient blood-brain barrier within the tumor.

The total lipid contents of all tumors in this study (Table 1) were lower than those of either human grey (6%) or white (16%) matter (23). Our values are similar to those of experimental astrocytomas grown subcutaneously, reported by Hauser, Eichberg, and Shein (26), but lower than those of some other studies of human primary intracranial tumors (24, 27). Results of analyses of the subcutaneously grown astrocytomas probably are more representative of the true composition of glial tumors because they are not contaminated with residual brain tissue which would elevate the lipid values.

The concentration of cholesterol in most of the tumors is closer to that of grey than white matter (Table 1). This is in keeping with the relatively high cholesterol concentration in myelin. However, as a percent of total lipids there is more cholesterol and less phospholipid in our series than experimental tumors grown either *in vivo* (26) or *in vitro* (28). These results may be due either to a fundamental difference

TABLE 1. Water, total lipid, cholesterol and total phospholipid P concentrations in neural tumors

	Water ^a	Total Lipid ^a	Cholesterol ^b	Total Phospholipid ^c	Number of Tumors Analyzed
Primitive neuroectodermal	86.7 ± .1	2.2 ± 0.6	21.8 ± 3.0	2.4 ± 0.2	4
Astrocytic					
All types	88.9 ± 3.0	2.4 ± 1.0	22.2 ± 3.8	2.6 ± 1.2	7
Well-differentiated	94.5	1.2	23.1	2.9	2
	90.4	1.6	20.0	3.0	
Anaplastic	87.1	4.1	25.8	1.1	2
	88.5	1.9	15.7	4.7	
Glioblastoma	87.2 ± 2.1	2.8 ± 0.6	23.5 ± 3.0	2.1 ± 0.7	3
Normal ^d					
Grey matter	81.9	5.9	22.0	2.8	
White matter	71.6	15.6	27.5	1.8	

^a Values expressed as percent fresh weight.

^b Values expressed as mg cholesterol per 100 mg lower phase lipid.

^c Values expressed as mg phospholipid P per 100 mg lower phase lipid.

^d Values from reference 23.

between the metabolism of human and animal tumors, or to differences in growth environments.

The percentage distribution of phosphorus among the major phospholipid classes for the tumor tissues is seen in **Table 2**. Inositol glycerophospholipid (IGP) and sphingomyelin (Sph) did not separate well in the solvent system used, so the values for both are combined. Most of the tumors had less serine glycerophospholipid (SGP) but all had more choline glycerophospholipid (CGP) than either grey or white matter. Ethanolamine glycerophospholipid (EGP) levels of most of the tumors were only slightly lower than normal brain. The high proportion of the IGP-sphingomyelin fraction and the low CGP content of white matter are due to myelin which is absent from tumor cells. However, not all tumors had lower proportions of IGP-sphingomyelin than normal brain. While the reason for this is not definitely established, our data from studies of cultured glioma cells (discussed below)

suggest that not all of the IGP-sphingomyelin fraction in tumor tissues is present in the tumor cells.

The concentrations of ganglioside sialic acid per 100 mg lower-phase lipids of all tumors (**Table 3**) were intermediate between those of normal human white and grey matter, 0.60 and 4.45 μmol per 100 mg lipid, respectively (23). On a dry weight basis the ganglioside concentrations of these tumors are higher than that of normal glia dissected from ox brain (29), subcutaneously grown hamster astroglial nodules (30), or both hamster and rat glia cultured in vitro (31). However, the ganglioside concentrations in the tumors are considerably lower than those of glial cells isolated by centrifugation from rabbit brain (32). Whether the ganglioside contents of human gliomas are higher or lower than those of the cells from which they originate must await more reliable estimates of the ganglioside composition of normal human glia.

The percentage distribution of sialic acid among

TABLE 2. Percentage distribution of phosphorus among major phospholipid classes

	SGP	IGP + Sph	CGP	EGP
Primitive neuroectodermal	7.3 ± 0.8	8.0 ± 2.5	55.1 ± 3.6	29.4 ± 1.4
Astrocytic				
All types	6.8 ± 3.7	16.6 ± 7.1	49.7 ± 6.9	27.0 ± 6.9
Well-differentiated	8.1	12.1	51.7	28.1
	13.3	11.3	46.5	28.8
Anaplastic	3.1	23.4	58.7	15.7
	4.2	8.1	57.7	30.1
Glioblastoma	6.4 ± 3.0	20.3 ± 7.0	44.5 ± 4.7	28.8 ± 8.2
Normal ^a				
Grey matter	12.2	13.7	39.0	31.7
White matter	16.7	18.6	27.8	31.9

^a Values are from reference 23.

the gangliosides of all the tumors is shown in **Table 4**. Because structural analyses have not been performed on any of these gangliosides, we prefer to name them according to chromatographic mobility using the nomenclature system of Korey and Gonatas (33). However, most cochromatograph with known standards isolated from normal human cerebral cortex, so the system of Svennerholm (34) is shown for those. In the solvent system employed for most of the tumors in this part of the study (chloroform-methanol-water-concentrated ammonium hydroxide 60:35:7:1), GD1a and GD3 do not separate well so their values have been combined as G3. The value for G-0 represents all of the sialic acid recovered between the origin and G-1.

Compared with normal human cerebral cortex, all tumors had increased proportions of G-5 and G-6 and smaller proportions of G-1 and G-2 (Table 4). Summing the proportions of G-5 and G-6 as one group and G-1, G-2, and G-3 as another, all tumors had increased proportions of the simple monosialogangliosides and decreased proportions of the di- and trisialogangliosides as a group. Indeed, the glioblastomas had the highest proportions of G-5 and G-6 and the differentiated astrocytomas had the lowest. Conversely, the glioblastomas had the lowest proportions of the di- and trisialogangliosides while the differentiated astrocytomas had the greatest. Our results are in agreement with those of Kostic and Bucheit (10) and Traylor and Hogan (35). Shochat, Abt, and Schengrund (36) found no correlation between histological grades and ganglioside patterns of human

TABLE 3. Ganglioside sialic acid in tumors

	Micromoles	
	Per Gram Fresh Weight	Per 100 mg Lower Phase Lipid
Primitive neuroectodermal	0.67 ± 0.25	2.86 ± 0.84
Astrocytic		
All types	0.46 ± 0.15	2.09 ± 0.67
Well-differentiated	0.29	2.38
Anaplastic	0.39	0.97
Glioblastoma	0.33	1.75
Glioblastoma	0.60 ± 0.13	2.23 ± 0.61
Normal grey matter ^a	2.6	4.45
Normal white matter ^a	0.36	0.60

^a Values are from reference 23.

neuroblastomas. However, the ganglioside patterns were more complex in neuroblastomas from patients who on clinical grounds were expected to have a good prognosis than from those with a poor prognosis. While direct comparisons are not possible because they did not quantitate specific gangliosides, their findings are in keeping with ours that more aggressive neural neoplasms have smaller proportions of di- and trisialogangliosides than less aggressive ones.

One possible interpretation of these results is that the tumor cells contain only G-5 and G-6 and the more complex gangliosides are present in residual brain tissue trapped by the infiltrating gliomas. The greater proportions of simple gangliosides would then

TABLE 4. Percentage distribution of sialic acid among gangliosides of primitive neuroectodermal and astrocytic tumors^a

	G0	G1 (GT)	G2 (GD1b)	G2a (GD2)	G3 (GD1a & GD3)	G4 (GM1)	G5 (GM2)	G6 (GM3)
Primitive neuroectodermal tumors								
Neuroblastoma	14.9	8.8	8.6	28.0	18.1	5.9	7.4	8.6
Medulloblastoma	8.9	8.7	6.2	15.2	13.3	6.2	17.8	23.8
Cerebral tumor	13.3	4.2	7.7	ND	17.9	4.0	51.1	2.1
Cerebral tumor	5.2	10.1	7.0	ND	31.6	23.6	11.0	11.7
Astrocytic tumors								
Differentiated	10.0	16.4	15.6	12.0	31.3	5.5	4.7	4.9
Anaplastic	8.5 ^c	8.7	5.8	9.7	43.3	6.6	13.8	3.9
Glioblastoma	2.2	3.4	3.2	ND	42.6	6.7	8.0	34.1
Glioblastoma	5.9	18.2	2.6	21.2	42.0	4.2	14.8	9.3
Glioblastoma	7.5	3.9	4.3	12.6	21.3	12.0	24.9	13.8
Glioblastoma	5.8	3.5	3.6	2.5	13.9	5.6	21.5	43.8
Glioblastoma	3.7 ^c	7.6	7.1	4.1	23.5	12.3	10.0	31.4
Normal grey matter ^b	3.9	19.7	16.7	3.0	40.0	14.2	1.7	1

^a Values are the percent of total sialic acid recovered from the plate present in each band.

^b Values are from reference 23.

^c Tumors subsequently cultured.

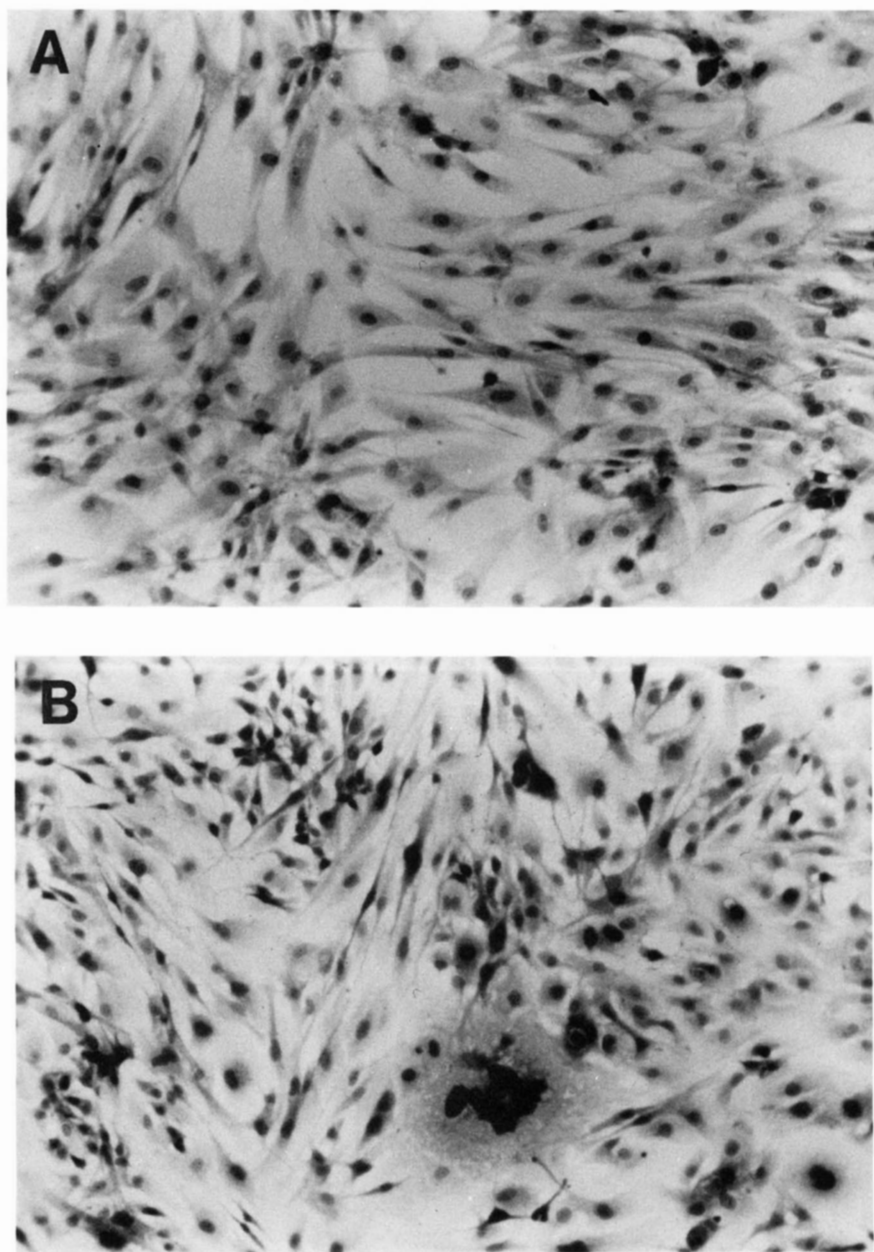


Fig. 1. *a.* Culture of astrocytoma at passage 7. Jacobi stain. Magnification $\times 495$. *b.* Culture of glioblastoma multiforme at passage 15. Jacobi stain. Magnification $\times 495$.

be due to a higher density of tumor cells and less trapped brain per volume of tissue analyzed. However, evidence against this interpretation comes from the results of our studies discussed below in which we cultured one astrocytoma and one glioblastoma multiforme in sufficient quantities to perform similar chemical determinations as were done on the tumor tissues.

The cultures of the astrocytoma were composed of cells with scanty to moderate amounts of cytoplasm (**Fig. 1a**). Many were bipolar but some were tri- and

multipolar. There was moderate nuclear pleomorphism but no multinucleate cells were seen. The cultures derived from the glioblastoma multiforme contained cells with variable amounts of cytoplasm. Many were spindle shaped but multipolar cells were also present (**Fig. 1b**). Marked nuclear pleomorphism and some multinucleate cells were present.

The total lipid contents on a dry weight basis of the two cultured tumors were similar to those of the tumor tissues from which they were grown (**Table 5**). The total phospholipid contents of both tumor cultures

were also similar to the tumor tissues. However, the concentrations of cholesterol on the basis of total lipid were approximately 35% less in both cultures than their respective parent tissues (Table 5). This may be due either to metabolic differences in the tumor cells as a consequence of tissue culture conditions or to cholesterol in a small amount of trapped myelin or myelin breakdown products of residual brain tissue. Further evidence supporting the latter interpretation is the smaller amount of the sphingomyelin-containing fraction and the larger amount of CGP in the cultured cells than in the tumor tissues (Table 5). As a proportion of total lipids, myelin has more cholesterol and sphingomyelin and less CGP than most other mammalian tissues.

The plasmalogen contents of the tissues and cultures (Table 5) are particularly interesting. Only trace amounts of choline plasmalogen are present in normal brain. However, our results show that between 2 and 4% of the total phospholipid of these tumors and cultures was choline plasmalogen. Furthermore, the tumors contained significant amounts of ethanolamine plasmalogen. Substantial amounts of both choline and ethanolamine plasmalogens have been found in whole tissues (37) and microsomes (38) of gliomas. Indeed, increases in alkyl and alkenyl ethers of glycerol have also been found in several non-neural human neoplasms (39). The similar plasmalogen contents of these tumor tissues and cultures indicate that results of *in vitro* investigations on this aspect of glioma metabolism may closely reflect their *in vivo* metabolism.

The ganglioside concentrations on the basis of total lipid weight were less in both cultures than in their respective parent tissues (Table 5). This may be ex-

TABLE 5. Lipid composition of tumor tissues and cultures

	Astrocytoma		Glioblastoma Multiforme	
	Tissue	Culture	Tissue	Culture
Total Lipid ^a	22.5	19.9	19.7	22.4
Cholesterol ^b	23.1	14.8	20.8	13.8
Phospholipid P				
Total ^c	2.90	2.16	2.63	2.80
Individual ^d				
SGP	8.1	7.0	6.2	4.5
IGP + Sph	12.1	4.2	17.1	6.3
CGP	49.7	60.4	35.3	52.8
CGP plas	2.0	3.6	3.8	4.2
EGP	13.5	12.3	17.1	16.9
EGP plas	14.6	12.2	20.3	14.6
Ganglioside sialic acid ^e	2.38	1.37	2.01	1.88

^a Percent of dry weight.

^b mg per 100 mg total lipid extract.

^c mg phosphorus per 100 mg total lipid extract.

^d Percent of total lipid phosphorus.

^e Micromoles per 100 mg total lipid extract.

TABLE 6. Ganglioside composition of tumor tissues and cultured cells^a

	Astrocytoma		Glioblastoma	
	Tissue	Culture	Tissue	Culture
G-7 (GM4)	0.0	0.0	0.0	3.1
G-6 (GM3)	3.9	17.6	31.4	16.4
G-5 (GM2)	13.8	8.1	10.0	41.6
G-4A	0.0	12.9	6.7	24.9
G-4 (GM1)	6.6	8.6	5.9	7.8
G-3A (GD-3)	33.7	31.4	13.7	3.5
G-3 (GD1a)	9.6	7.6	9.8	0.0
G-2A (GD2)	9.7	7.1	4.1	2.7
G-2 (GD1b)	5.8	3.8	7.0	1.2
G-1 (GT)	8.7	0.5	7.6	0.0
G-0	8.5	2.4	3.7	1.9

^a Values are the percent of total sialic acid recovered from the plate present in each band.

plained by the presence in the tissues of a few ganglioside-rich structures, such as axons, which did not grow in culture. However, it is also possible that there was a change in ganglioside metabolism of the tumor cells due to the culture conditions. The ganglioside concentrations of both cultures were much lower than those of normal human cerebral cortex, and higher than those of white matter (Table 2). These results are similar to those for subcutaneously grown hamster astroglial nodules (30), rat astrocytomas (40), hand-dissected ox glial clumps (29), and both rat glioma and mouse neuroblastoma cells cultivated *in vitro* (41). Our cultures (Table 5) contained about twice the ganglioside concentration of cultured hamster astroglia, rat astroblasts (31), or human skin fibroblasts (41).

The ganglioside patterns of both cultures were complex (Table 6). In the chromatographic system used for this part of the study some bands, such as GD3, run as doublets (42) which were pooled for quantitative analyses. Both cultures contained small amounts of gangliosides which cochromatographed with two of the four major normal human brain gangliosides (G-2, G-4) as well as larger amounts of the minor ones (G-2a, G-3a, G-5, and G-6). G-3 was lacking from the glioblastoma culture, and a ganglioside (G-4a) with a chromatographic mobility not found in normal brain or the astrocytoma tissue was present in significant amounts in both of the cultures and in the glioblastoma tissue (Fig. 2). These results are consistent with those of Manuelidis and Manuelidis (44) who found complex patterns of gangliosides in some cultures of human glioblastomas, but different from those of Dufford et al. (43), who found GM3 to comprise 95% of the ganglioside in Cox Clone human glioblastoma cells. There are at least three possible explanations for these differences. First, it is possible that the tumor from which the Cox Clone was derived

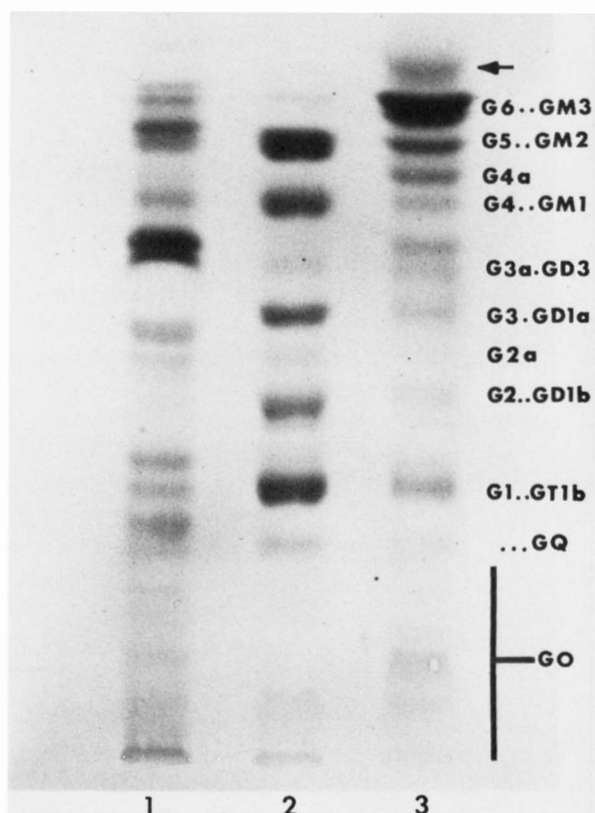


Fig. 2. Thin-layer chromatogram of gangliosides run in solvent system chloroform-methanol-0.02% calcium chloride in water 50:40:10 on silica gel (Merck) plates and sprayed with resorcinol reagent. Lane 1, astrocytoma tumor tissue; lane 2, normal human cerebral cortex ganglioside standards plus added GM-2; lane 3, glioblastoma multiforme tumor tissue. The arrow indicates a spot that is not resorcinol positive. Both tumors are those that were cultured and whose lipid compositions are in Tables 5 and 6.

was composed of a variety of cells, all of which had a much simpler ganglioside composition than the cells of our tumors. Second, it is possible that the tumor was composed of a variety of neoplastic cell types, some with complex and others with simple ganglioside patterns, and that the Cox Clone was derived from the latter. Third, it has been found that, with extended time in culture, the ganglioside patterns of human glioblastoma cultures become simple (44, 45). Thus it is possible that at earlier passages the Cox Clone cells may have been complex but have simplified with time. None of the cultures we examined were past the 15th passage, which is relatively early and obviously before the process of ganglioside pattern simplification had occurred.

In general, compared with their parent tissues, the cultures in this study contained less of the more slowly moving gangliosides with decreased proportions, to varying degrees, of all those with mobilities slower

than that of G-4. With the exception of decreases in the G-5 of the astrocytoma and G-6 of the glioblastoma, all of the gangliosides with mobilities faster than that of G-3a were greater in the cultured cells than in their parent tissues (Table 6). Our results indicate that even highly malignant glioma cells have complex patterns of gangliosides although different from normal brain. Manuelidis and Manuelidis (44) and Manuelidis, Yu, and Manuelidis (45) have found changes in the ganglioside composition of human glioblastoma cells with extended time in tissue culture. Those that had simple patterns and low levels of gangliosides had changed their morphology in culture to a polygonal shape. However, others maintained higher levels and more complex patterns of gangliosides, as well as their original morphology with multiple cytoplasmic processes. Neither of our cultures became polygonal and the ganglioside concentrations were slightly higher than those reported by Manuelidis and Manuelidis (44). Our observations are in agreement with their suggestion that the ganglioside composition of cells may be related to their morphology.

In summary, the results of our ganglioside analyses of 11 human neural tumors show several findings of interest. All of the tumors had ganglioside concentrations intermediate between normal human grey and white matter but the ganglioside patterns were different than normal brain. They all had increased proportions of G-5 and G-6 with correspondingly lower proportions of G-1, G-2, and G-3. The rapidly growing glioblastoma multiforme had the highest proportions of G-5 and G-6, and the more slowly growing astrocytomas had the lowest proportions of these two gangliosides. This indicates that the more rapidly growing tumors have a greater shift in their ganglioside composition of the simpler ones. However, small amounts of more complex gangliosides were present in the neoplastic cells of both an astrocytoma and a glioblastoma multiforme cultured in vitro, showing that there is not a complete shift to the production of simple gangliosides in these neoplastic cells. ■

This investigation was supported by American Cancer Society, Ohio Chapter; by American Cancer Society grant 1n-16P; and by grant 5 P30 CA 16058-3 awarded by NCI, DHEW.

Manuscript received 5 July, 1978; accepted 6 November 1978.

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