We have recently reported the percentage of fatty acid distribution in primary brain tumors (11) and meningiomas (10). In these tumors a marked increase in polyunsaturated fatty acids, in particular linoleic acid, was found. In addition, preliminary studies on the distribution of phenylhydantoin suggested that in vitro lipid solubility of the drug may represent an index of penetrability in primary gliomas (6). Since extensive drug testing on a screening basis is not possible clinically, we set out to determine the fatty acid patterns in a mouse, which had a subcutaneous or intracerebral transmissible glial tumor.

**MATERIAL AND METHODS**

Two types of ependymoma were obtained from Dr. W. H. Sweet, Department of Neurosurgery, Massachusetts General Hospital. These tumors included the Sloan-Kettering glioma which represents a transmissible tumor (originally induced by methylcholanthrene in C57 mice) and the Roswell Park Perese glial tumor (also originally induced by methylcholanthrene in C3H mice). A colony of each of these glial tumors was established by appropriate S.C. transplantation. In S.C. transplants, the perese tumor grew to an approximate average of 2 cm in diameter, while the Sloan-Kettering glioma #26 grew to an approximate average of 1.5 cm in diameter, in 2 weeks.

The percentage of fatty acid distribution in the following transmissible gliomas was determined in six perese S.C. gliomas, two intracerebral perese gliomas, and six SK S.C. gliomas. Results were compared with those obtained from the cerebral gray matter of four C3H mice and four C57 control mice. The amount of each of five major lipids and the fatty acids in each lipid were analyzed in four perese gliomas.

**Total fatty acid analysis by GLC.**—Utilizing a method established previously (1), the methyl esters of fatty acids in fresh tissues were obtained by direct methylation of the total lipids. From 25 to 100 mg of brain tissue of transmissible glioma were placed in 15 ml of a solution of 98% methyl alcohol and 2% concentrated sulfuric acid by volume, and refluxed for 2 hr. This solution was washed with 10 ml of distilled water and 10 ml of n-hexane and then shaken. The water layer was transferred to another separatory funnel and washed with 10 ml of n-hexane. The water layer was discarded. The two n-hexane layers were combined and washed 3 times with distilled water. This solution was filtered through 1 gm anhydrous sodium sulfate (Na₂SO₄) into a 125-ml Erlenmeyer flask. The sodium sulfate was washed with 5 ml of n-hexane. The total n-hexane filtrate was evaporated on a water respirator to obtain proper concentration of methyl esters to be analyzed by gas-phase chromatography. Although other lipids soluble in n-hexane were present in the filtrate, in addition to the methyl esters of fatty acids, they created no significant interference upon introduction into the column for analysis.

The methyl esters were analyzed on a Barber-Colman gas chromatograph, model no. 10. Separations were carried out by the use of diethylene glycol succinate polyester, 15.4% by weight, on 80-100 mesh, acid washed Chromsorb W., supplied by Applied Science Laboratories, Inc., State College, Pa. The column was maintained at 170°C, and the rate of flow or argon was 2.5 ml/sec. The total running time for each prepared sample was 120 min.

The machine was standardized daily with a National Institutes of Health methyl ester standard. Reproducible results, within 1.5% of actual composition, were obtained. Each fatty acid peak was identified by comparison of retention times with methyl ester standards of myristic, palmitic, stearic, palmitoleic, oleic, linoleic, arachidonic, eicosapentaenoic, and docosahexaenoic fatty acids, which were supplied by Applied Science Laboratories, Inc.
### TABLE 1

**Percentage of Distribution of Total Fatty Acids and Their Standard Deviation in Transmissible Gliomas, Subcutaneous and Intracerebral**

<table>
<thead>
<tr>
<th>No. of Tumors</th>
<th>C14*</th>
<th>X1</th>
<th>C16</th>
<th>C16,1</th>
<th>X1</th>
<th>C18</th>
<th>C18,1</th>
<th>C18,2</th>
<th>C20,4</th>
<th>C20,5</th>
<th>C22,6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracerebral Pnxse</td>
<td>2</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 1.1</td>
<td>28.5 ± 3.4</td>
<td>3.6 ± 0.3</td>
<td>0.5</td>
<td>14.7 ± 0.1</td>
<td>27.0 ± 7.7</td>
<td>6.5 ± 0.3</td>
<td>9.3 ± 3.0</td>
<td>1.3 ± 1.0</td>
</tr>
<tr>
<td>Subcutaneous Pnxse</td>
<td>6</td>
<td>1.6 ± 0.3</td>
<td>2.2 ± 0.7</td>
<td>25.9 ± 1.7</td>
<td>3.8 ± 0.5</td>
<td>Tr.</td>
<td>14.1 ± 1.7</td>
<td>20.8 ± 2.7</td>
<td>10.1 ± 1.3</td>
<td>14.1 ± 2.8</td>
<td>3.2 ± 2.6</td>
</tr>
<tr>
<td>Subcutaneous SK</td>
<td>6</td>
<td>1.5 ± 0.1</td>
<td>2.2 ± 0.7</td>
<td>23.4 ± 2.7</td>
<td>3.1 ± 0.5</td>
<td>Tr.</td>
<td>14.5 ± 1.3</td>
<td>23.7 ± 4.1</td>
<td>8.8 ± 1.3</td>
<td>9.4 ± 2.8</td>
<td>5.1 ± 3.0</td>
</tr>
<tr>
<td>Normal brain Pnxse</td>
<td>4</td>
<td>Tr.</td>
<td>2.7 ± 2.6</td>
<td>32.6 ± 2.6</td>
<td>Tr.</td>
<td>2.8 ± 1.4</td>
<td>20.6 ± 1.6</td>
<td>14.0 ± 0.9</td>
<td>0.7 ± 0.2</td>
<td>9.0 ± 1.1</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>Normal brain SK</td>
<td>4</td>
<td>Tr.</td>
<td>2.9 ± 1.0</td>
<td>28.7 ± 4.0</td>
<td>0.5</td>
<td>4.0 ± 0.8</td>
<td>20.4 ± 0.5</td>
<td>14.2 ± 1.0</td>
<td>0.8 ± 0.3</td>
<td>8.5 ± 1.2</td>
<td>1.4 ± 0.7</td>
</tr>
</tbody>
</table>

* The abbreviations used are: C14, myristic; C16, palmitic; C16:1, palmitoleic; C18, stearic; C18:1, oleic; C18:2, linoleic; C20:4, arachidonic; C20:5, eicosapentenoic; C22:6, docosahexenoic fatty acids. X1 and X2 were tentatively identified as palmitaldehyde and stearaldehyde. Tr., trace.

### TABLE 2

**Percentage of Distribution of Fatty Acids and Their Standard Deviation of 5 Major Lipids in 4 Pnxse Transmissible Gliomas**

<table>
<thead>
<tr>
<th>C14*</th>
<th>X1</th>
<th>C16</th>
<th>C16,1</th>
<th>X1</th>
<th>C18</th>
<th>C18,1</th>
<th>C18,2</th>
<th>C20,4</th>
<th>C22,6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fatty acids</td>
<td>5.0 ± 1.0</td>
<td>—</td>
<td>36.2 ± 3.3</td>
<td>7.1 ± 2.3</td>
<td>—</td>
<td>14.4 ± 3.6</td>
<td>23.2 ± 4.1</td>
<td>8.1 ± 0.6</td>
<td>4.9 ± 2.2</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.7 ± 0.5</td>
<td>—</td>
<td>25.4 ± 2.3</td>
<td>6.4 ± 1.2</td>
<td>—</td>
<td>4.4 ± 1.3</td>
<td>43.7 ± 1.6</td>
<td>18.4 ± 0.6</td>
<td>—</td>
</tr>
<tr>
<td>Phosphatidyl-ethanolamine</td>
<td>1.0 ± 0.3</td>
<td>14.2 ± 0.1</td>
<td>7.7 ± 2.1</td>
<td>Tr.</td>
<td>4.6 ± 0.6</td>
<td>22.2 ± 3.7</td>
<td>14.1 ± 1.5</td>
<td>4.5 ± 0.8</td>
<td>19.4 ± 2.5</td>
</tr>
<tr>
<td>Phosphatidyl-inositol</td>
<td>—</td>
<td>—</td>
<td>6.9 ± 2.1</td>
<td>—</td>
<td>—</td>
<td>51.2 ± 4.1</td>
<td>9.3 ± 0.7</td>
<td>4.5 ± 0.7</td>
<td>23.1 ± 2.6</td>
</tr>
<tr>
<td>Lechitin</td>
<td>2.4 ± 0.5</td>
<td>1.3 ± 0.5</td>
<td>40.0 ± 0.8</td>
<td>4.1 ± 1.2</td>
<td>—</td>
<td>15.6 ± 1.4</td>
<td>18.0 ± 1.6</td>
<td>8.0 ± 0.8</td>
<td>8.9 ± 1.6</td>
</tr>
</tbody>
</table>

* For explanation of abbreviations, see Table 1.
The peaks on each chromatogram were designed as follows: C14 (myristic acid), C16 (palmitic), C16:1 (palmitoleic), C18 (stearic), C18:1 (oleic), C18:2 (linoleic), C20:4 (arachidonic) C20:5 (eicosapentenoic), and C22:6 (docosahexenoic).

Further identification was carried out by bromination of the methyl esters and precipitation of aldehydes by 2,4-dinitrophenylhydrazine (8). These aldehydes were designated X1 and X2 and were tentatively identified as palmitaldehyde and stearaldehyde, respectively, by inference from the relative time of retention.

The percentile composition of fatty acids was calculated by measuring the area under each peak by triangulation and equating the sum of the areas to 100%.

Separation of neutral and phospholipids.—The tissue was homogenized in 20 volumes of chloroform-methanol (2:1, v/v) for 3 min. in a micro Waring Blender, filtered through Whatman no. 3 paper under slight vacuum, and washed with 50 ml of the above solvent. To the filtrate was added 0.2 volume of 0.73% NaCl. The mixture was stirred, and the aqueous layer was separated by centrifugation (2). The aqueous layer was aspirated and washed 4 times successively with chloroform-methanol-0.73% NaCl (3:48:47, by volume) before the total lipid extract was concentrated to dryness and taken up in light petroleum ether. Insoluble material was removed and the extract was concentrated to a small volume (10–15 ml).

Separation into neutral and phospholipids was made on a 10-gm silicic acid column plus 5 gm of Hyflo SuperCel. The total lipid extract was placed on this column and eluted first with 200 ml of chloroform and then with 200 ml of absolute methanol. Aliquots of the chloro-
form and methanol fractions were taken to determine the amount of neutral lipids and phospholipids present, respectively.

**Thin layer chromatography of neutral lipids.**—Thin layer plates were prepared by mixing a slurry of silica gel G and water, 1:2, and spreading an even layer 0.25 mm thick on 20 × 20 cm glass plates using a mechanical spreader. The plates were dried at 100°C for 1 hr. before use.

Standard solutions of approximately 1 mg/ml of cholesterol, cholesteryl stearate, triolein, dipalmitin, and palmitic acid in chloroform were prepared. Various amounts of these standards (from 0.2 to 10 μg) were spotted on silicic acid plates, and developed in an ascending manner, using n-hexane, diethyl ether, and glacial acetic acid (80:20:3, by volume) for 40 min.

Standard cholesterol esters, triglycerides, free fatty acids, and cholesterol for thin layer chromatography were obtained from Applied Science Laboratories, Inc. Silicic acid and organic chemicals of analytical reagent grade were obtained from Mallinckrodt Chemical Works. Silica gel G for thin layer chromatography was obtained from Research Specialties Company.

The plates were sprayed with chromic sulfuric acid (a saturated solution of K₂Cr₂O₇ in 87% H₂SO₄) and heated at 180°C for 35 min. After cooling, the charred spots were measured on a Photovolt densitometer. As reported by Mangold (3) and Privett et al. (5), the areas under the densitometer curves are directly proportional to the amount of sample. Privett and Blank (4) recommended the use of chromic-sulfuric acid instead of 50% sulfuric acid as the charring agent. They found that mono-, di-, and triglycerides give spots of equal intensities after correction for differences in their carbon densities, and that unsaturation per se had no effect on the yield of carbon. However, a standard curve for the major components was obtained for each plate.

**Thin-layer chromatography of phospholipids.**—The phospholipids were separated on thin layer by the method of Skipski et al. (9) with developing solvent of chloroform-methanol-glacial acetic acid-water (50:25:7:3, by volume). Sphingo-myelin, lecithin, phosphatidyl inositol, phosphatidyl ethanolamine, and polyglycerolphosphatides were identified and compared with the phospholipids from liver.

After lipids were separated, they were detected with 2,7-dichlorofluorescein; the individual lipids were scraped from the thin-layer plaques and eluted with methanol. Fatty acid esterification was done with 2% H₂SO₄ in methanol and refluxed for 1 hr. Following this, lipids were extracted with n-hexane (1).

**RESULTS**

The results and standard deviations of the fatty acid distribution in transmissible glial tumors and predominantly gray matter are recorded in Table 1. The fatty acid pattern of normal brain tissue is very different from tumor tissue, especially in C18:2, linoleic acid (0.7% vs. 9%) and C22:6, docosahexaenoic acid (13% vs. 4%) (Chart 1). In Table 2 the distributions and standard deviations of the fatty acids of major lipids in the Perese glioma are reported. It appears that each lipid has a fatty acid pattern, characteristic of that lipid in each of the four tumors analyzed. Table 3 represents the quantitative data of five major lipids in the Perese glioma. The triglycerides represent the major lipid material in the tumor.

**DISCUSSION**

The percentage of fatty acid distribution after total lipid extraction of tumors is a relatively gross screening procedure for lipid patterns. In general the patterns of each of the Perese tumors and of each of the Sloan-Kettering tumors were similar (Table 1). These patterns, furthermore, were comparable to the fatty acid patterns of primary human glial tumors previously described (11). In the same fashion the fatty acid patterns of the host mice cerebral gray matter of C3H and C57 mice were similar. The patterns of the control tissues were sharply different from that of the tumor, especially in the percentage of C18:2 and C22:6. The fatty acid patterns of the subcutaneous gliomas did not appear to be altered by intracerebral growth. This suggests that the basic lipid patterns are related to the primary independent biology of the tumor and not to the location in the host. In the fractionation of total lipids performed, it was apparent that the fatty acids attached to the individual lipids were characteristic of each lipid. Although the quantitative data indicated that triglycerides were the major lipid material in tumors, these lipids occur only in measurable amounts in normal cerebral gray matter (7). These differences also suggest a basic difference in the tumor lipid biosynthesis.

**REFERENCES**

5. **Privett, O. S.; Blank, M. L.; and Lundberg, W. O.** Deter-


Fatty Acid Analysis of Two Experimental Transmissible Glial Tumors by Gas-Liquid Chromatography

Arthur A. Stein, Edward Opalka and I. Rosenblum

Cancer Res 1965;25:201-205.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/25/2_Part_1/201

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.