Phospholipid Content of Mitochondrial and Microsomal Membranes from Morris Hepatomas of Varying Growth Rates

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ABSTRACT

Mitochondria and microsomal membranes were prepared from normal, fetal, and regenerating rat liver and from seven Morris hepatomas of varying growth rates and analyzed for percentage of phospholipid composition and phospholipid content per mg protein. The Morris hepatomas studied included the 7777, 5132tc, 7800, 7794A, 7787, 9633, and 9618A.

Cardiolipin is localized in mitochondria in all tissues studied. The sphingomyelin percentage and content are increased in mitochondria of fetal rat liver and more rapidly growing hepatomas, 7777, 5123tc, and 7794A but are similar to those of normal liver in hepatomas with slower growth rates. The ratio of the percentage of phosphatidylethanolamine to that of phosphatidylcholine in mitochondria and microsomes is not substantially altered in most of the seven hepatomas and in the rapid growth controls. Our studies do not support the concept of equalization of membrane phospholipid composition in hepatomas with regard to the subcellular localization of cardiolipin and the ratios of percentage of phosphatidylethanolamine to that of phosphatidylcholine. However, our results confirm the increased percentage of sphingomyelin in the membranes of hepatomas with very rapid growth rates.

Substantial changes in the membrane phospholipid content per mg of protein are present in the seven Morris hepatomas. The mitochondrial phospholipid content per mg of protein is substantially increased in nearly all of the hepatomas and in fetal liver but appears to be normal in regenerating rat liver. Microsomal phospholipid per mg of protein is considerably decreased in nearly all of the hepatomas and in the rapid growth controls. Changes in membrane phospholipid content per mg of protein appear to be of a greater quantitative significance in these Morris hepatomas than are alterations in the percentage of phospholipid composition of the respective membrane fractions.

INTRODUCTION

Mitochondrial and microsomal membranes from the Zajdela hepatoma and from Hepatomas 27, 22, and 48 have been reported to have an altered distribution of phospholipid compared with that found in normal or regenerating rat liver (1, 2). The specific phospholipid distribution characteristic for these membranes in normal liver is reported to be altered so that the percentage of phospholipid composition of mitochondria and microsomes is "equalized"; this has been termed "chemical dedifferentiation" of tumor cell membranes (2). Specifically, the changes which have been reported are: (a) an increased percentage of cardiolipin in tumor microsomes; (b) an increased percentage of sphingomyelin in tumor mitochondria; and (c) alteration of the usually distinctive ratio of the percentage of phosphatidylethanolamine to that of phosphatidylcholine in microsomes and mitochondria. The magnitude of the equalization of phospholipid composition is reported to have a positive correlation with the degree of malignancy of the tumor (1). Recently, Dyatlovitskaya et al. (3) have proposed that the chemical dedifferentiation of tumor cell membranes is the result of the action of a universal lipid exchange protein which is present in the pH 5.1 supernatant of Hepatoma 27 but is not present in the pH 5.1 supernatant from normal liver.

However, other investigators have not been able to completely confirm the findings of dedifferentiation of membrane phospholipids in hepatomas. Feo et al. (4) did not find a significant percentage of cardiolipin in microsomes in the Morris 5123 hepatoma, and the mitochondrial percentage of sphingomyelin was only slightly increased. However, this hepatoma is a minimal-deviation tumor. In the poorly differentiated Morris 7777 hepatoma, we did not find significant amounts of cardiolipin in the microsomes; the percentage of sphingomyelin in the mitochondria was elevated, but only slight changes were noted in the ratio of percentage of phosphatidylethanolamine to percentage of phosphatidylcholine in 7777 membranes compared with that of normal liver (10). Generally, similar results were reported in Morris 7777 hepatoma by Reitz et al. (16). Morton et al. (14) and Waite et al. (19) did not find a significant percentage of cardiolipin in 7777 hepatoma microsomes and reported a mitochondria sphingomyelin percentage similar to that of normal liver. In contrast to our results (10) and the data of Reitz et al. (16), these authors found some evidence for equalization of the percentage of phosphatidylethanolamine to that of phosphatidylcholine in 7777 mitochondria and microsomes (14, 19). There was a considerable increase in 7777 mitochondrial phospholipid content per mg of protein, while the phospholipid content of 7777 hepatoma microsomes was substantially reduced (10, 16, 19). We found no change in the amount of protein per mitochondrion, indicating that there is an absolute increase in the phospholipid per mitochondrion (11). However, similar conclusions cannot be drawn for 7777 microsomes, since the decreased phospholipid to protein ratio can be explained either by increased microsomal protein or by decreased microsomal phospholipid.

To further define the nature of subcellular organelle membrane phospholipid aberrations in hepatomas, we studied the phospholipid composition and content per mg of protein of mitochondria and microsomes from 7 Morris hepatomas of varying growth rates and degrees of malignancy, and from

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normal liver and 2 nonmalignant, rapidly growing controls, fetal and regenerating rat liver. The results of these investigations are the subject of this publication.

MATERIALS AND METHODS

Tumor Passage and Preparation of Subcellular Fractions. Morris hepatomas were maintained in Buffalo rats by i.m. passage to the hind legs. The approximate length of time after passage required for the respective hepatomas to reach 6 to 8 g is: 7777 and 5123tc, 3 to 4 weeks; 7800 and 7794A, 9 to 14 weeks; and 7787, 9618A, and 9633, 9 to 11 months. The rats ranged in age from 3 to 12 months at the time of sacrifice. Our previous study has shown that the length of time that 7777 hepatomas are maintained in the host animal (size range, 0.5 to 33.0 g) has no effect on the phospholipid content of mitochondria or microsomes (10). Similarly, these parameters do not change in the liver of adult Buffalo or Sprague-Dawley rats ranging in weight from 100 to 800 g (age, 1 to 12 months).5

Regenerating rat liver was prepared as described by Higgins and Anderson (7). Fetal rat livers were obtained from female Buffalo rats in their 18th to 20th day of pregnancy. Liver tissue or hepatoma was removed from the rat after an overnight fast. The respective tissues were rinsed in ice-cold 0.25 M sucrose containing 5 mM Tris-HCl (pH 7.4) and 2 mM EDTA, minced, and rinsed several times. A 10% w/v homogenate was prepared by 3 or 4 strokes of a motor-driven Potter-Elvehjem homogenizer. Microsomes and sucrose gradient-purified mitochondria were prepared as described previously (10). Protein was measured by the method of Lowry et al. (13).

Assay Procedures. Subcellular fractions were assayed for purity by the use of marker enzymes. Succinate dehydrogenase was assayed according to the method of Green et al. (6). Rotenone-insensitive NADPH-cytochrome c reductase was assayed according to the method of Sottacasa et al. (18). The recovery of succinate dehydrogenase and NADPH-cytochrome c reductase from the homogenates ranged from 94 to 104% and 86 to 103%, respectively. Lipid extracts were prepared and washed by the method of Folch et al. (5). Lipid phosphorus determinations were made according to the method of Rouser et al. (17).

Thin-Layer Chromatography. Two-dimensional thin-layer chromatography of the lipid extracts from the respective subcellular fractions was performed with 0.25-mm layers of Silica Gel H prepared with magnesium acetate. The silica slurry contained 90 g of Silica H, 5 g of magnesium acetate, and 240 ml of distilled water. After spreading, the plates were air-dried and activated at 120° for 60 min, and approximately 400 nmol of phospholipid phosphorus were applied to the origin. The plates were developed in the first dimension with chloroform/methanol/concentrated ammonia/water (60/30/2/1.5, by volume). The plates were dried for 30 min in an argon or nitrogen atmosphere followed by development in the second dimension with chloroform/acetone/methanol/glacial acetic acid/water (3/4/1/0.5, by volume). The lipids were located with iodine vapors, and the areas corresponding to the individual lipids were scraped into tubes for phosphorus analysis.

Chemicals. Cytochrome c and NADPH were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Silica Gel H was obtained from EM Laboratories, Elmsford, N. Y. Other chemicals were of analytic reagent grade from usual commercial sources. Chloroform and methanol were redistilled prior to use.

RESULTS

Table 1 shows the percentage of phospholipid composition of mitochondria from nonmalignant liver and from Morris hepatomas of varying degrees of malignancy. The percentage of cardiolipin was slightly decreased in mitochondria from fetal liver and several of the more rapidly growing hepatomas (7777, 7800, and 7794A) relative to that of normal liver mitochondria. In the well-differentiated hepatomas (7787, 9633, and 9618A), the percentage of cardiolipin was essentially normal. In the hepatomas, the percentage of phosphatidylcholine and phosphatidylinositol in the mitochondria did not differ substantially from that of normal liver mitochondria. The percentage of phosphatidylethanolamine in mitochondria was somewhat increased in hepatomas 7800, 7794A, 7787, and 9633. In fetal liver and in the most rapidly growing hepatomas (7777 and 5123tc), the percentage of sphingomyelin in mitochondria was increased. In the hepatomas with intermediate growth rates (7800 and 7794A) and slow growth rates (7787, 9633, and 9618A) the percentage of sphingomyelin in mitochondria did not differ significantly from that of normal liver. Fetal liver mitochondria had an increased percentage of phosphatidylserine.

As shown in Table 2 the microsomal preparations did not contain substantial percentages of cardiolipin. The microsomal percentage of phosphatidylethanolamine was variable in the hepatomas, increased in many (5123tc, 7800, 7794A, 7787, and 9633), but was essentially normal in others (7777 and 9618A). The phosphatidylcholine percentage of total lipid phosphorus was generally slightly reduced or normal in the hepatomas. The percentage of sphingomyelin in microsomes from fetal liver and from tumors of rapid (7777 and 5123tc) or intermediate growth rates (7800 and 7794A) was significantly increased over that of normal liver. However, minimal-deviation hepatomas had normal percentages of sphingomyelin in the microsomes. The percentage of phosphatidylinositol was reduced in fetal microsomes and tended to be normal or reduced in hepatomas; no definite correlation with tumor growth rates was apparent. Increases in the percentages of phosphatidylserine were noted in fetal liver and in some hepatomas, but no definite correlation with tumor growth rate was noted.

In contrast to the relatively minor changes in the percentage of phospholipid composition of tumor membranes noted above, Table 3 shows that the phospholipid content of tumor membranes per mg of protein differs substantially from that found in the corresponding membranes of normal liver. The mitochondrial phospholipid content of fetal liver and all of the hepatomas, with the exception of the 9633, was increased over that of normal liver and regenerating liver. These increases were statistically significant and represented in some cases as much as a 78 to 89% increase over the content of phospholipid in normal liver mitochondria. In contrast, the microsomal phospholipid content relative to protein was decreased in all hepatomas (except the 9618A) and in fetal and regenerating liver.

Results are expressed as percentage of total lipid phosphorus ± S.D. The numbers in parentheses represent the number of preparations studied. Small amounts of lysophosphatidylcholine, phosphatidic acid, bis(monoacylglycerol)phosphate, and other unidentified trace phospholipids were present in the preparations above and together accounted for 0.31 to 4.0% of lipid phosphorus. These have been omitted from the tabulation, accounting for the fact that the sum of the percentages is less than 100. Recoveries of lipid phosphorus were greater than 95%. The nomenclature used for the phospholipids is not meant to indicate the exclusive presence of diacyl forms. In the absence of analytical data, we recognize the possible presence of alkyl and alk-1-ethyl forms.

Table 1

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Normal liver (6)</th>
<th>Fetal liver (3)</th>
<th>Regenerating liver (1)</th>
<th>7777 hepatoma (7)</th>
<th>5123tc hepatoma (3)</th>
<th>7800 hepatoma (3)</th>
<th>774A hepatoma (3)</th>
<th>7787 hepatoma (3)</th>
<th>9633 hepatoma (3)</th>
<th>9618A hepatoma (3)</th>
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</thead>
<tbody>
<tr>
<td>Cardiolipin</td>
<td>17.2 ± 2.0</td>
<td>12.1 ± 3.6a</td>
<td>16.7 ± 0.8b</td>
<td>15.7 ± 1.4</td>
<td>14.8 ± 0.9g</td>
<td>14.1 ± 1.5a</td>
<td>15.4 ± 1.4</td>
<td>14.9 ± 3.4</td>
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<tr>
<td>Phosphatidylethanolamine</td>
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<td>34.1 ± 1.1</td>
<td>36.1 ± 2.6b</td>
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<td>39.8 ± 3.6a</td>
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<td>35.5 ± 1.4</td>
<td>31.5 ± 9.9</td>
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<td>Phosphatidylinositol</td>
<td>2.3 ± 1.0</td>
<td>4.2 ± 0.9a</td>
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<td>Phosphatidylserine</td>
<td>7.9 ± 0.7</td>
<td>5.6 ± 0.8a</td>
<td>5.3 ± 1.2</td>
<td>6.0 ± 0.6b</td>
<td>6.9 ± 1.2</td>
<td>8.4 ± 3.4</td>
<td>5.4 ± 0.6b</td>
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<tr>
<td>Phosphatidylserine</td>
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<td>4.4 ± 1.6a</td>
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</tbody>
</table>

a < p < 0.05 versus normal liver.
b < p < 0.024 versus normal liver.
c < p < 0.001 versus normal liver.
d < p < 0.005 versus normal liver.

Table 2

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Normal liver (6)</th>
<th>Fetal liver (3)</th>
<th>Regenerating liver (1)</th>
<th>7777 hepatoma (7)</th>
<th>5123tc hepatoma (3)</th>
<th>7800 hepatoma (3)</th>
<th>774A hepatoma (3)</th>
<th>7787 hepatoma (3)</th>
<th>9633 hepatoma (3)</th>
<th>9618A hepatoma (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiolipin</td>
<td>0.6 ± 0.6</td>
<td>0.3 ± 0.6</td>
<td>0.6 ± 0.6</td>
<td>0.4 ± 0.5</td>
<td>0.2 ± 0.3</td>
<td>1.6 ± 2.7</td>
<td>0.8 ± 0.7</td>
<td>0.5 ± 0.8</td>
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<tr>
<td>Phosphatidylethanolamine</td>
<td>22.5 ± 1.2</td>
<td>23.7 ± 1.0</td>
<td>28.4 ± 0.4</td>
<td>25.4 ± 0.1b</td>
<td>27.7 ± 0.4b</td>
<td>26.8 ± 1.9b</td>
<td>25.4 ± 1.1b</td>
<td>31.0 ± 2.9b</td>
<td>23.8 ± 6.1</td>
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<tr>
<td>Phosphatidylcholine</td>
<td>55.1 ± 3.5</td>
<td>54.1 ± 0.9</td>
<td>53.6 ± 2.6</td>
<td>46.7 ± 1.4b</td>
<td>49.7 ± 1.5d</td>
<td>50.6 ± 1.1d</td>
<td>53.4 ± 0.9</td>
<td>43.8 ± 5.6c</td>
<td>56.4 ± 5.0</td>
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<tr>
<td>Phosphatidylinositol</td>
<td>3.9 ± 1.2</td>
<td>6.3 ± 0.1a</td>
<td>3.8 ± 0.4</td>
<td>13.4 ± 2.0b</td>
<td>10.0 ± 0.5b</td>
<td>4.9 ± 0.2b</td>
<td>5.3 ± 1.2</td>
<td>4.8 ± 1.1</td>
<td>4.2 ± 0.8</td>
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</tr>
<tr>
<td>Phosphatidylserine</td>
<td>12.1 ± 0.7</td>
<td>8.6 ± 0.5a</td>
<td>8.7 ± 1.2</td>
<td>9.5 ± 0.6b</td>
<td>10.9 ± 0.4a</td>
<td>9.9 ± 0.4b</td>
<td>9.4 ± 0.3a</td>
<td>10.2 ± 0.7a</td>
<td>11.3 ± 2.8</td>
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<tr>
<td>Phosphatidylserine</td>
<td>3.5 ± 0.5</td>
<td>6.6 ± 0.2a</td>
<td>3.4 ± 0.8</td>
<td>5.9 ± 1.7d</td>
<td>4.8 ± 0.4e</td>
<td>4.5 ± 0.7e</td>
<td>3.6 ± 0.8</td>
<td>5.7 ± 1.4d</td>
<td>3.2 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

a < p < 0.005 versus normal liver.
b < p < 0.001 versus normal liver.
c < p < 0.01 versus normal liver.
d < p < 0.024 versus normal liver.
ea < p < 0.005 versus normal liver.

The decreases in phospholipid per mg of protein tended to be greatest in the tumors with rapid or intermediate growth rates (7777, 5723tc, 7800, and 7794a) and represented decreases ranging from 28 to 44%.

The mitochondrial and microsomal membrane content of the individual phospholipids relative to protein was calculated from the percentage of phospholipid composition and the lipid phosphorus content per mg of membrane protein. Table 4 shows the lipid phosphorus content of mitochondria from various sources. The cardiolipin content of mitochondria was increased in nearly all of the hepatomas (44.1 to 63.0 nmol lipid phosphorus per mg) compared with that found in normal liver (34.1). Cardiolipin levels were normal in the 7800 and 9633 hepatomas and in fetal and regenerating rat liver. Substantial increases in the phosphatidylcholine and phosphatidyethanolamine content of mitochondria were noted in all hepatomas and in fetal rat liver. These increases ranged from about 30 to almost 100% relative to normal liver mitochondria. Mitochondrial sphingomyelin was increased from 4.5 nmol lipid phosphorus per mg in normal liver to 11.0 in fetal liver, 15.7 in the 7777 hepatoma, 14.5 in the 5123tc hepatoma, and 9.0 in the 7794A hepatoma. The mitochondrial levels of sphingomyelin in regenerating rat liver were normal. Some hepatomas had normal (7800, 7787, and 9618A) or reduced levels (9633) of sphingomyelin in the mitochondria. Statistically significant increases in phosphatidylinositol were found in mitochondria from the 7777, 5123tc, and 7794A hepatomas. Phosphatidylserine levels in mitochondria were generally comparable to that of normal liver except in fetal rat liver where a substantially elevated level was found.

Table 5 shows the lipid phosphorus content of microsomes from various sources. Cardiolipin is not present in significant amounts in any of the respective microsomal preparations. In agreement with other reports, substantial reductions in the microsomal content of phosphatidyethanolamine and phosphatidylcholine were found in the 7777 hepatoma (10, 16, 19). Similar results were obtained for the other rapidly growing hepatoma (5123tc) and for the hepatomas of intermediate growth rates.
growth rates (7800 and 7794A). One well-differentiated hepatoma, the 9633, also had reduced levels of phosphatidylcholine and phosphatidylethanolamine in microsomes, while the 7787 and 9618A hepatomas had normal or slightly increased levels. Fetal liver microsomes also contained reduced amounts of these phospholipids. Phosphatidylinositol levels were reduced significantly in fetal liver microsomes and in all hepatomas except the 9618A. Phosphatidylserine levels in tumor microsomes were generally within the range found in microsomes from normal liver.

In contrast to the reductions in the microsomal content of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, the amount of sphingomyelin per mg of microsomal protein was increased in the 2 rapidly growing hepatomas (7777 and 5123tc). Normal microsomes contained 18.3 nmol sngomerylin per mg compared with 32.3 in the 7777 hepatoma and 34.5 in the 5123tc hepatoma (Table 5). The sphingomyelin content of microsomes from hepatomas of intermediate or slow growth rates and from fetal liver was within the normal range for liver microsomes.

Table 6 shows the ratio of the percentage of phosphatidylethanolamine to that of phosphatidylcholine in mitochondria and microsomes calculated from the data in Tables 1 and 2. This ratio was: normal liver mitochondria, 0.97; fetal liver, 0.81; and regenerating rat liver, 1.06. Most of the hepatomas had ratios of percentage of phosphatidylethanolamine to that of phosphatidylcholine in the range of the nonmalignant controls with the exception of the 7794A (1.23) and the 9633 (1.24). Similarly, this ratio in microsomes from the nonmalignant controls ranged from 0.41 to 0.53; all hepatomas had essentially normal ratios with the exception of the 9633 minimal-deviation hepatoma (0.71). Thus, it appears that these 7 Morris hepatomas generally retain their distinctive ratios of percentage of phosphatidylethanolamine to that of phosphatidylcholine in mitochondria and microsomes.

No correlation was found between the lipid composition and content and the recovery of succinate dehydrogenase and rotenone-insensitive NADPH-cytochrome c reductase in the purified mitochondrial and microsomal fractions from normal liver or hepatomas, respectively. Thus, it appears that the
Table 5
Phospholipid content and marker enzyme activity of microsomes

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Normal liver (6)</th>
<th>Fetal liver (3)</th>
<th>Regenerating liver (1)</th>
<th>7777 hepatoma (7)</th>
<th>5123tc hepatoma (3)</th>
<th>7800 hepatoma (3)</th>
<th>7794A hepatoma (3)</th>
<th>7787 hepatoma (3)</th>
<th>9633 hepatoma (3)</th>
<th>9618A hepatoma (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiolipin</td>
<td>2.7 ± 2.9b</td>
<td>0</td>
<td>1.1</td>
<td>1.3 ± 1.3</td>
<td>1.2 ± 1.7</td>
<td>0.5 ± 0.8</td>
<td>5.0 ± 8.7</td>
<td>3.5 ± 3.0</td>
<td>1.6 ± 2.8</td>
<td>0</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>105.0 ± 6.0</td>
<td>66.6 ± 2.2b</td>
<td>92.5</td>
<td>48.6 ± 6.4b</td>
<td>87.0 ± 2.3b</td>
<td>76.4 ± 1.2b</td>
<td>85.6 ± 4.6b</td>
<td>115.0 ± 10.0</td>
<td>127.0 ± 4.6b</td>
<td>133.0 ± 34.0</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>257.0 ± 18.0</td>
<td>152.0 ± 6.0b</td>
<td>174.0</td>
<td>103.0 ± 14.0b</td>
<td>160.0 ± 9.0b</td>
<td>137.0 ± 4.0b</td>
<td>162.0 ± 5.9b</td>
<td>242.0 ± 33.0</td>
<td>170.0 ± 22.0b</td>
<td>331.0 ± 19.0b</td>
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<tr>
<td>Sphingomyelin</td>
<td>18.3 ± 5.6</td>
<td>24.2 ± 1.1b</td>
<td>28.4</td>
<td>27.8 ± 6.7b</td>
<td>32.7 ± 1.4b</td>
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<td>41.9 ± 2.6</td>
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<td>Phosphatidylinositol</td>
<td>16.5 ± 2.3</td>
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<td>14.6 ± 5.6</td>
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<td>8.6 ± 2.1b</td>
<td>14.4 ± 2.6</td>
<td>16.4 ± 5.2</td>
<td>22.9 ± 3.8</td>
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<tr>
<td>Phosphatidylserine</td>
<td>41.9 ± 9.2</td>
<td>7.2 ± 3.3</td>
<td>27.6</td>
<td>9.8 ± 0.6</td>
<td>10.2 ± 0.9</td>
<td>15.0 ± 0.2</td>
<td>36.5 ± 5.0</td>
<td>35.6 ± 12.6</td>
<td>10.3 ± 2.3</td>
<td>61.4g</td>
</tr>
</tbody>
</table>

Succinate dehydrogenase

\[
\text{Ratio of percentage of phosphatidylcholine to percentage of phosphatidylethanolamine} = \frac{\text{Percentage of phosphatidylcholine}}{\text{Percentage of phosphatidylethanolamine}}
\]

‎\* Mean ± S.D.

\* p < 0.001 versus normal liver.

\* p < 0.01 versus normal liver.

\* p < 0.05 versus normal liver.

\* p < 0.05 versus normal liver.

\* Specific activity (nmol/mg/min).

\* Average of 2 determinations.


**DISCUSSION**

This study of 7 Morris hepatomas of varying growth rates is generally not in agreement with the concept of equalization of membrane phospholipid composition as proposed by others (1-3). We do not find significant amounts of cardiolipin in the microsomal preparations with the degree of contamination of the microsomal preparations with mitochondria based on succinate dehydrogenase. Furthermore, as shown in Tables 1, 2, and 6, there is generally a retention of the distinctive phospholipid composition of these 7 Morris hepatomas generally retain their characteristic percentage of phospholipid composition.

As shown in Table 3, there is no obvious correlation between the degree of increase in mitochondrial phospholipid content, while the fetal liver (266, versus phospholipid content of normal liver (11, 15, 20). Fetal liver content of normal liver mitochondrial protein, has been shown to be an artifact caused by decreased mitochondrial protein, while in contrast to the results of normal liver mitochondria (11, 15, 20). Fetal liver mitochondrial phospholipid content is significantly lower than that of normal liver mitochondria (11, 15, 20).

On the other hand, most of the 7 hepatomas studied showed a decrease in mitochondrial phospholipid content, which is consistent with the degree of increase in mitochondrial phospholipid content, while the fetal liver mitochondrial phospholipid content is significantly lower than that of normal liver mitochondria (11, 15, 20).

The results cannot be explained by the isolation of selected populations of mitochondria from the various tissues.

![Table 6](Downloaded from canrecs.aacrjournals.org on August 13, 2017. © 1979 American Association for Cancer Research.)

<table>
<thead>
<tr>
<th>Source</th>
<th>Normal liver</th>
<th>Hepatomas</th>
<th>Mitochondria</th>
<th>Microsomes</th>
<th>Mitochondria %</th>
<th>Microsomes %</th>
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of protein). Microsomal phospholipid content is reduced significantly in all hepatomas except the minimal-deviation 9618A hepatoma, in general agreement with previous reports (4, 10, 16, 19). Microsomes from fetal liver and regenerating rat liver also exhibit a decreased phospholipid content. However, in contrast to the situation with mitochondria, we cannot exclude the possibility that the low ratio of phospholipid to protein observed in tumor microsomes may be due to an increased content of microsomal protein.

The increased phospholipid content of hepatoma mitochondria is the result of a generalized increase in all phospholipid relative to protein, while in microsomes the decreased phospholipid content in tumors and in fetal and regenerating rat liver can be accounted for by a decreased content of phosphatidylcholine and phosphatidylethanolamine. The microsomal sphingomyelin content of hepatomas is increased in the most rapidly growing tumors and is normal in those with less rapid growth rates. The studies of Waite et al. (14) in 7777 hepatoma microsomes indicate that decreases in the phospholipid deacylation-reacylation cycle may be an important factor in the reduced microsomal phospholipid content. In addition, our previous studies demonstrate a decreased activity of CDP-choline:1,2-diacylglycerol cholinephosphotransferase in the microsomes of the 7777 hepatoma as compared with that of normal liver microsomes (11).

The molecular basis for the increased phospholipid content of hepatoma mitochondria is unclear. In fact, the mechanism of mitochondrial acquisition of membrane phospholipid in normal tissues is also poorly understood. Mitochondria are capable of synthesizing cardiolipin de novo (8, 9, 11) but lack the enzymes required for the synthesis of phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and sphingomyelin (11, 21, 22). In mitochondria from several Morris hepatomas, the activity of CTP:phosphatidate cytidylyltransferase is increased substantially while the activity of other mitochondrial enzymes of cardiolipin synthesis is similar to that found in normal liver (11). The moderate increases in hepatoma mitochondrial cardiolipin content reported in this paper can, in all probability, be accounted for by the increased activity of CTP:phosphatidate cytidylyltransferase which appears to be rate limiting in de novo cardiolipin biosynthesis (11). Morton et al. (14) found that the calcium-stimulated phospholipase A activity of 7777 hepatoma mitochondria against [3H]phosphatidylethanolamine was "more readily expressed" than the same activity in liver organelles but concluded that the maximal activity in the 7777 hepatoma against [3H]phosphatidylethanolamine was lower than that of normal liver. However, we found no essential difference between normal liver and the 7777 hepatoma in the rate of hydrolysis of endogenous [32P]cardiolin or phosphatidylcholine by mitochondrial phospholipase A

References


Phospholipid Content of Mitochondrial and Microsomal Membranes from Morris Hepatomas of Varying Growth Rates

Karl Y. Hostetler, Bruce D. Zenner and Harold P. Morris

Cancer Res 1979;39:2978-2983.

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