The Lipid Composition of Hibernoma as Compared with That of Lipoma and of Mouse Brown Fat

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SUMMARY

The lipid composition of 2 hibernomas was compared with that of 4 ordinary lipomas and of mouse brown fat. The concentrations of cholesterol and phospholipids, as compared to total fat, were higher in the hibernomas and the brown fat than in the lipomas. The phospholipid composition of the hibernomas, 2 of the lipomas, and the brown fat was determined using thin-layer chromatography; it was found that whenever the brown fat differed from the lipomas—with an increase or a decrease—the hibernomas did so too. The results are in agreement with the opinion that hibernoma arises from brown fat.

MATERIALS AND METHODS

Two patients were operated on for a soft tissue tumor in the thigh. In both cases, histologic examination showed a hibernoma. The 2 hibernomas thus obtained were used in the present study; they have been described in detail elsewhere (1). In addition, the material comprised 4 ordinary lipomas from 4 different patients, and brown fat from the interscapular fat bodies of 8 male adult white mice; the brown fat was pooled in 2 groups, each consisting of 4 samples. Before the chemical analysis, the hibernomas and the lipomas were fixed in 10 % formaldehyde (formalin) solution.

For extraction of the lipids, about 0.5 gm of each tissue was placed in chloroform:methanol (2:1), disintegrated with a glass rod, and left in the mixture at room temperature for 1 hr. Then extraction and partition were completed as described by Folch et al. (4). Cholesterol (3), lipid phosphorus (9), and triglyceride (2) concentrations were determined on the extract. For comparison between the various tissues, the lipids could not be related to dry weights of whole tissue, because the vessels of the hibernomas had been perfused with barium sulphate for microangiographic studies (1). Instead the lipids were related to protein: the dry weight of total lipids extracted from a sample of tissue was related to the amount of soluble protein found in the same sample. Soluble protein was determined by the method of Lowry et al. (6).

Phospholipids were separated by thin-layer chromatography on silica gel (7). This was free from calcium sulphate binder (Kieselgel H nach Stahl, Merck), and the chromatograms were developed in chloroform:methanol:water (65:25:4) at +4°C. until the solvent front had moved a suitable distance (usually 30 min.). On each plate the sample to be tested was applied centrally with human plasma lipids on one side and nothing on the other side. The plasma sample served as a standard for identification of spots which were made visible by iodine vapors. Seven spots were recognized on the chromatogram of the plasma sample (7): the front region; a faint spot just below the front; spots containing cephalins, lecithin, sphingomyelin, and lyssolecithin; and the origin. Areas corresponding to these spots were marked on the unknown sample, and then the silica gel was carefully scraped off these areas into test tubes for lipid phosphorus determination. Digestion was performed in the presence of silica gel. Corresponding areas of the same size on the empty side served as blanks. Recovery of applied lipid phosphorus was about 80 % for all samples analyzed. No further attempt to identify the phospholipids was made because the main interest was in the relative distribution of the spots in question. The phospholipid composition was determined in only 2 of the 4 ordinary lipomas.

RESULTS AND DISCUSSION

Table 1 shows the lipid composition of the hibernomas, lipomas, and brown fat. The amounts of cholesterol and phospholipids, as determined in relation to total fat, were higher in the hibernomas and the brown fat than in the
Other hand, the concentration of lysolecithin, as well as that of sphingomyelin, was lower in the hibernomas and lipomas. The spot below the front gave a Phospholipids named after corresponding spots obtained from human plasma phospholipids run as control. Composition is given in % of total phospholipids. Table 2 shows the distribution of phospholipids in the hibernomas, lipomas, and mouse brown fat. Lipids given in % of total fat, obtained by addition of cholesterol, phospholipids (estimated as 25 × lipid phosphorus value), and triglycerides (estimated as triolein). Total lipids in relation to soluble protein given as dry weight of total lipids extracted from a sample whose content of soluble protein was determined.

### Table 1

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Hibernomas</th>
<th>Lipomas</th>
<th>Brown Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>0.16</td>
<td>0.18</td>
<td>0.23</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>0.42</td>
<td>1.38</td>
<td>0.21</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>99.4</td>
<td>98.4</td>
<td>99.8</td>
</tr>
<tr>
<td>Total lipids (mg) per mg soluble protein</td>
<td>414</td>
<td>312</td>
<td>518</td>
</tr>
</tbody>
</table>

* Lipids given in % of total fat, obtained by addition of cholesterol, phospholipids (estimated as 25 × lipid phosphorus value), and triglycerides (estimated as triolein). Total lipids in relation to soluble protein given as dry weight of total lipids extracted from a sample whose content of soluble protein was determined.

### Table 2

| Phospholipid Composition of Hibernomas, Lipomas, and Mouse Brown Fat |
|---|---|---|---|
| Fraction No. | Phospholipids | Hibernomas | Lipomas | Brown Fat |
| 1 | Front (polyglycerophospholipids?) | 2.1 | 4.3 | 1.7 | 1.0 | 9.0 | 6.5 |
| 2 | Spot below front (unknown) | 2.2 | 3.2 | 1.7 | 1.3 | 6.3 | 4.2 |
| 3 | Cephalins | 13.3 | 14.9 | 9.6 | 10.5 | 10.5 | 8.4 |
| 4 | Lecithin | 50.6 | 48.3 | 65.3 | 61.4 | 50.0 | 52.9 |
| 5 | Sphingomyelin | 12.3 | 6.8 | 12.4 | 14.2 | 7.8 | 10.2 |
| 6 | Lysolecithin | 8.9 | 13.2 | 4.3 | 5.6 | 6.3 | 9.4 |
| 7 | Origin | 10.6 | 9.3 | 5.0 | 6.0 | 10.1 | 8.4 |

* Phospholipid fractions named after corresponding spots obtained from human plasma phospholipids run as control. Composition is given in % of total phospholipids.

## References

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