COMPARATIVE STUDIES ON THE LIPID COMPOSITION OF SOME DIGENETIC TREMATODES

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(Received 10 January 1975)

Abstract—YUSUF A. N. K. & SIDDIQI, A. H. 1976. Comparative studies on the lipid composition of some digenetic trematodes. International Journal for Parasitology 6: 5-8. Neutral lipids and phospholipids of six digenetic trematodes, Cotylophoron cotylophorum, Gastrothylax crumenifer and Gigantocotyle explanatum from water buffalo, Echinostoma malayanum and Fasciolopsis buski from pig and Isoparorchis hyselobagri from cat-fish were analyzed. Total lipid concentrations in trematodes varied considerably irrespective of their hosts and habitats. While triglycerides were the major components in all species, considerable amounts of phospholipids and free fatty acids were present in all species. Cholesterol was minimum (4-9%) in more or less all species, except in F. buski, where cholesterol, phospholipids and triglycerides constituted 13-14% and free fatty acids around 7%. Among phospholipids, choline and ethanolamine phosphatides were major polar lipids. Sphingomyelin and cardiolipin were present as small fractions and lysophosphatidylcholine was evenly distributed among all the species (9-12%) except in F. buski, which contained a little higher content (15%).

INDEX KEY WORDS: Total lipids; triglycerides; cholesterol; phosphatidylcholine; cephalins; sphingomyelin; Cotylophoron cotylophorum; Gastrothylax crumenifer; Gigantocotyle explanatum; water buffalo; Fasciolopsis buski; Echinostoma malayanum; pig; Isoparorchis hyselobagri; cat-fish.

INTRODUCTION

Knowledge of total lipid content or lipid fractions of digenetic trematodes is quite fragmentary. Although information concerning the nature and occurrence of lipids in other helminths is rapidly accumulating, trematode lipids have remained a more or less neglected subject (von Brand, 1973). There is, however, a paucity of any comparative work in this field of trematode biochemistry.

The present work is based on the investigations carried out on the following species of digenetic trematodes living in different habitats in three different hosts: Cotylophoron cotylophorum, Gastrothylax crumenifer, both from the rumen, and Gigantocotyle explanatum from the liver of the Indian water buffalo, Bubalus bubalis L.; Fasciolopsis buski and Echinostoma malayanum from the small intestine of the pig, Sus scrofa; and Isoparorchis hyselobagri from the swim bladder of the catfish, Wallago attu. This choice of trematodes for the present work offered an opportunity to examine not only species differences, but also to see if the type of habitat and/or host species have any influence on lipid composition of the digenetic trematodes.

MATERIALS AND METHODS

Adult C. cotylophorum, G. crumenifer and G. explanatum were obtained from the rumen and liver of the water buffalo from the abattoir and brought to the laboratory in warm 0-9% sodium chloride solution. Adult E. malayanum and F. buski were collected from the small intestine of the pigs slaughtered at the Central Dairy Farm, Aligarh and I. hyselobagri were obtained from the swim bladder of the cat-fish. Prior to lipid extraction, mammalian trematodes were washed several times and kept at 37°C in normal saline, fish trematodes were kept in modified Ringer (Forster & Taggart, 1950) at 25°C. Lipids were extracted with chloroform-methanol (2:1, v/v) by the method of Misra (1968) and washed with a NaCl solution according to Folch, Lees & Sloane-Stanley (1967). The extract was evaporated to dryness in vacuo, and the residue made to volume with chloroform. Total lipids of the wet parasites were determined by drying and weighing suitable aliquots of the lipid extracts. Total cholesterol and phospholipids were determined by the method of Bloor, Pelkan & Allen (1922) and Marinetti (1962) respectively. Triglyceride concentrations were determined as glycerol after alkaline hydrolysis according to Van Hanel & Zilversmit (1957). Free fatty acids (FFA) were determined colorimetrically (Itaya & Ui, 1965).

Phospholipids were further fractionated by ascending TLC (Skipski, Peterson & Barclay, 1964) using chloroform-methanol-water (65:25:4, v/v/v) as the solvent system. The phospholipid bands were visualized by iodine vapour and eluted with N-methanolic-HCl and their concentrations were determined as described above.

The various fractions were identified by comparing the Rf values with standard phospholipids applied on the same plate.
RESULTS

The total lipid content in the 6 species of trematodes of mammals and fish was quite variable and ranged between 10 and 50% of the dry weight of worms (Table 1). Analysis of the lipid components revealed striking differences among trematode species. They consisted of around 4-14% cholesterol, 8-32% phospholipids, 13-53% triglycerides and 5-17% FFA. Besides, a high percentage of unidentified lipids (35-68%) is also evident except in C. cottiophorum and I. hypselobagri where the content ranged between 12 and 19% only.

Phospholipid analysis showed that the phospholipid composition in general varied little among all the species under study (Table 2). Phosphatidylincholine and mixture of phosphatidylethanolamine, serine and inositol (cephalins) were the major polar lipids, i.e. 31-47% and 12-24%, respectively. The concentrations of sphingomyelin, cardiolipin and lysophosphatidylincholine were low among all the species and ranged between 3 and 19%, 1 and 6% and 9 and 15%, respectively.

As can be seen from Table 1 some rather striking differences existed between the lipid classes of trematodes from similar host and/or habitat. More marked is the difference in cholesterol and phospholipid contents in C. cottiophorum and G. crumenifer, and F. buski and E. malayanum, however, the concentration of these parameters in G. explanatum was very much similar to that of G. crumenifer. Furthermore, the content of triglycerides, one of the major lipid components in all the species, and FFA of trematodes from cattle rumen and pig intestine were relatively similar. Gigantocotyle explanatum, however, does not contain as much triglycerides and FFA though it lives in liver, a habitat which is considered to be the chief site of lipid synthesis. Isoparorchis hypselobagri contains more than 50% triglycerides of the total lipid while about 9-14% cholesterol, phospholipids and FFA. This also indicates that the concentration of neutral lipids is much higher than that of polar lipids in this trematode.

DISCUSSION

It can be seen from Table 1 that all trematodes differ considerably in their total lipid content. In C. cottiophorum, G. explanatum and I. hypselobagri the total lipid accounts for one third of the dry weight.

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**Table 1—Total lipid and lipid fractions of some digenetic trematodes**

<table>
<thead>
<tr>
<th>Species</th>
<th>Total lipids % of fresh tissues (Mean ± S.E.M.)</th>
<th>Lipid fractions % of dry tissues</th>
<th>Phospholipids</th>
<th>Triglycerides</th>
<th>Free fatty acids</th>
<th>Cholesterol</th>
<th>Unidentified lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. cottiophorum</td>
<td>8.57 ± 0.39</td>
<td>27.3</td>
<td>32.1</td>
<td>24.8</td>
<td>16.5</td>
<td>7.5</td>
<td>19.1</td>
</tr>
<tr>
<td>G. crumenifer</td>
<td>3.30 ± 0.11</td>
<td>10.5</td>
<td>19.5</td>
<td>24.8</td>
<td>15.2</td>
<td>4.8</td>
<td>35.7</td>
</tr>
<tr>
<td>G. explanatum</td>
<td>7.08 ± 0.05</td>
<td>34.4</td>
<td>16.3</td>
<td>19.1</td>
<td>9.3</td>
<td>5.1</td>
<td>50.2</td>
</tr>
<tr>
<td>E. malayanum</td>
<td>9.65 ± 0.16</td>
<td>39.0</td>
<td>8.5</td>
<td>14.0</td>
<td>5.0</td>
<td>4.1</td>
<td>68.4</td>
</tr>
<tr>
<td>F. buski</td>
<td>8.88 ± 0.21</td>
<td>50.4</td>
<td>13.9</td>
<td>13.2</td>
<td>6.9</td>
<td>14.0</td>
<td>52.0</td>
</tr>
<tr>
<td>I. hypselobagri</td>
<td>2.43 ± 0.09</td>
<td>29.5</td>
<td>14.4</td>
<td>52.6</td>
<td>10.9</td>
<td>9.2</td>
<td>12.9</td>
</tr>
</tbody>
</table>

The results for lipid fractions are expressed as means of % of total lipids for 4 separate determinations.

**Table 2—Phospholipid fractions of some digenetic trematodes**

<table>
<thead>
<tr>
<th>Species</th>
<th>Unknown at origin</th>
<th>Lysophosphatidylcholine</th>
<th>Sphingomyelins</th>
<th>Phosphatidylcholine</th>
<th>Cephalins</th>
<th>Cardiolipin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. cottiophorum</td>
<td>15.7</td>
<td>8.9</td>
<td>3.3</td>
<td>47.4</td>
<td>24.0</td>
<td>2.6</td>
</tr>
<tr>
<td>G. crumenifer</td>
<td>20.9</td>
<td>12.2</td>
<td>9.6</td>
<td>31.3</td>
<td>20.3</td>
<td>5.5</td>
</tr>
<tr>
<td>G. explanatum</td>
<td>12.2</td>
<td>11.2</td>
<td>4.2</td>
<td>45.9</td>
<td>23.6</td>
<td>2.8</td>
</tr>
<tr>
<td>E. malayanum</td>
<td>12.1</td>
<td>11.8</td>
<td>5.5</td>
<td>46.0</td>
<td>18.2</td>
<td>6.3</td>
</tr>
<tr>
<td>F. buski</td>
<td>17.0</td>
<td>15.3</td>
<td>5.0</td>
<td>40.7</td>
<td>19.1</td>
<td>3.0</td>
</tr>
<tr>
<td>I. hypselobagri</td>
<td>17.1</td>
<td>10.6</td>
<td>18.9</td>
<td>39.4</td>
<td>12.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

The results are expressed as means of % of total phospholipids for 4 separate determinations.
of worms, while in *G. crumenifer*, *E. malayanum* and *F. buski* it is 10%, 39% and 50%, respectively. Obviously these parasites can be divided into two groups, i.e. worms with high lipid content (7-10%) and low lipid content (2-3%) on the wet weight basis. There may be several factors influencing the lipid content of a worm, for example water content, type of habitat, and species differences.

Goil (1958, 1964) reported the total lipid content of *G. crumenifer* and *G. explanatum*, but his values are low and are not at all comparable with our observations, neither on the dry nor on the wet weight basis. The disparity may be due to the different techniques employed. He extracted total lipids with ether which is not considered to extract lipid completely.

The total lipid content of *G. crumenifer* and *I. hypselobagri* can be compared with those of *F. gigantica* (Goil, 1958) and *F. hepatica* (Weinland & von Brand, 1926) only on the wet weight basis, whereas on the dry weight basis, the total lipid content in all species studied except *G. crumenifer*, *E. malayanum* and *F. buski* was found to be as high as recently reported for *S. mansoni* by Smith & Brooks (1969). Furthermore, the total lipid content, as well as the content of polar lipids and cholesterol, is not identical in *F. buski* and *E. malayanum*, both from pig intestine; similarly it is not identical in *G. crumenifer* and *C. cotylophorum*, from the rumen of water buffalo. However, the triglycerides and FFA are present in approximately similar amounts. Nonetheless, it can be seen that the habitat, at least in these cases, has no influence on lipid content in trematodes. Smith, Brooks & Lockard (1970) and Meyer, Meyer & Bueding (1970) have recently reported that trematodes do not synthesize cholesterol and long-chain fatty acids, obtaining these substances from the host to synthesize other lipid complexes.

Fractionation of lipids showed that triglycerides and phospholipids account for a high percentage of the total lipids along with a considerable amount of FFA (Table 1). Cholesterol makes up the minor lipid component in all species except *F. buski*. Interestingly, *I. hypselobagri* contains quite a high level of triglycerides (52.6%). This may be due to the fact that it inhabits an atypical habitat—the swim bladder, which has biologically significant amounts of oxygen (Siddiqi & Nizami, 1974) which is known to be required for the biosynthesis of unsaturated fatty acids in other animals (Goldfine & Bloch, 1963). The low lipid content of *I. hypselobagri* may be due to the high water content of this fish trematode, which is 92% according to Siddiqi, Islam & Nizami (1975). High water content in tissues reduces the lipid solubility most probably in the case of unsaturated fats. High percentages of triglycerides have also been reported for *S. mansoni* (Smith & Brooks, 1969; Meyer et al., 1970), another trematode which lives in an oxygen rich habitat.

The content of other lipid fractions, however, are quite comparable with those reported earlier for *G. crumenifer* (Goil, 1964) and *S. mansoni* (Smith & Brooks, 1969). The phospholipid composition has not so far been studied in any trematode but major phospholipid groups, identical to those reported here and also present in free-living animals, have been demonstrated in *S. mansoni* eggs (Smith, Lucia, Doughty & von Lichtenberg, 1971). As shown in Table 2, phosphatidylcholine is the dominant fraction (31-47%), whereas relatively low concentrations of sphingomyelin (3-19%) and cardiolipin (1-6%) are present in all the species. Furthermore, phosphatidylethanolamine, serine and inositol (cephalins) are present in significant quantities (12-24%) but less than that of phosphatidylcholine. Lysophosphatidylcholine is evenly distributed in all the species. The differences between the various phospholipid fractions in all parasites are less pronounced. However, *I. hypselobagri* contains a larger amount of sphingomyelin than the other species and lower levels of cephalins, cardiolipin and lysophosphatidylcholine. Comparatively the phospholipid composition of *C. cotylophorum* and *G. crumenifer* and *E. malayanum* and *F. buski* differs in all fractions in the former pair while that of the latter merely differed in lysophosphatidylcholine, phosphatidylcholine and cardiolipin levels although each pair of trematodes live in the same habitat.

The literature shows that the unsaponifiable material of various groups of helminths (including nematodes and trematodes) contain sterols other than cholesterol (von Brand, 1928, 1933, 1939, 1957; Salisbury & Anderson, 1939). Recently some cholesterol was also found in *E. revolutum* and *S. mansoni* (Smith & Brooks, 1969; Barrett, Cain & Fairbairn, 1970), however, cholesterol was the major sterol present. As trematodes are not able to synthesize sterols, the presence of any cholesterol may be of the dietary origin and hence the composition of the environment may deeply influence its nature and relative abundance (Barrett et al., 1970; Meyer et al., 1970). The unsaponifiable fractions are also reported to contain substances other than sterols or ascorbates as in the case of *Ascaris*. These include hydrocarbons, waxes, soaps and higher alcohols (von Brand, 1959). Therefore, higher values of unidentified lipids present in all species except *C. cotylophorum* and *I. hypselobagri* may be due to the above compounds, whose presence appears to be necessary for the survival in the habitat with pH differing markedly from neutrality.

Identification of these unknown lipid compounds could lead to a better understanding of the lipid metabolism of trematodes.

Acknowledgements—The authors are grateful to the I.C.A.R. for financial support and to Head, Department
of Zoology, Professor W. Rahman, and Professor A. M. Siddiqi of the Department of Chemistry for providing laboratory facilities.

REFERENCES


