LIPIDS OF THE CYSTICERCI OF *TAENIA HYDATIGENA* (CESTODA)

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(Received 7 February 1973)

Abstract—1. The Cysticerci of *Taenia hydatigena* were separated into tissue and fluid fractions. The phospho- and neutral lipids of each fraction were determined.

2. The phospholipids isolated were lysolecithin, sphingomyelin, lecithin, phosphatidyl inositol, phosphatidyl serine and cephalin. Phosphatidyl inositol was the most abundant.

3. Thirteen free as well as bound fatty acids were identified and quantitatively estimated by GLC. Even carbon and saturated fatty acids were the most prevalent. Palmitic and stearic acids displayed the highest concentrations in all determinations.

4. An appreciable degree of unsaturation was also observed in free and bound fatty acids. The significance of this finding is discussed.

INTRODUCTION

DURING the past few years investigators have been preoccupied with the identification and quantitation of lipids from helminth parasites. Elaborate techniques including the application of column, thin-layer and gas–liquid chromatographies to the identification of lipids, have made possible an increase in the knowledge of different classes of lipids and also of various components in each class.

The most important non-taeniid cestodes that have been extensively studied for the characterization of phospho- and neutral lipids in their tissues are *Moniezia expansa* (Totterman & Kirk, 1939), *Raillietina cesticillus* (Reid, 1941, 1942; Botero & Reid, 1969), *Hymenolepis diminuta* and *H. citelli* (Warren, 1957; Warren & Daugherty, 1957; Fairbairn et al., 1961; Harrington, 1965; Ginger & Fairbairn, 1966a, b; Kilejian et al., 1968; Overturf & Dryer, 1968), *Poeleoncristium caryophyllum* *Dasyrynchus giganteus*, *Thysanocephalum thysanocephalum*, *Grillotia simmonsi*, *Lacistorhynchus tenuis*, *Orygmatobothrium musteli* and *Gariobothrium verticillatum* (Buteau et al., 1969, 1971).

Most of the work on the lipid composition of the taeniid tapeworms, except for some studies performed on the adult *Taenia saginata* (Smorodinstev & Bebeshin, 1939; Čmelík & Bartl, 1956), has been confined to the larval stages. The lipid chemistry of the larval tapeworm *Cysticercus fasciolaris* revealed that the lipids were rich in phospholipids, cholesterol, cerebrosides and fatty acids which consisted mainly of palmitic, stearic and arachidic (Salisbury & Anderson, 1939).
Early studies on the lipid composition of hydatid cysts of *Echinococcus granulosus* demonstrated the presence of acetic, propionic, valeric, succinic and higher fatty acids in the cyst fluid (Flössner, 1924, 1925; Coutelen, 1931). Čmelik (1952a, b) reported a low lipid content of the cyst membrane of *E. granulosus* consisting chiefly of cholesterol. Cholesterol was also demonstrated to be the only sterol present in hydatid cysts and derived completely from the host's pool of sterols (Frayha, 1968). Digenis *et al.* (1970) identified seventeen fatty acids in the scolices of *E. granulosus* with C₁₈:₁, C₁₈:₀, C₂₀:₀, C₂₀:₄ being the most significant. Recently Frayha (1971) observed that the cysticerci of *T. hydatigena* as well as the scolices of the other two taeniid cestodes, *E. granulosus* and *E. multilocularis*, could synthesize some lipids from acetate. The only lipid identified was cholesterol which was not biosynthesized from acetate but was probably assimilated from the host's pool of sterols.

The basic tissue organization of the cysticercus (larval stage) of *Taenia hydatigena* is probably similar to other cysticerci belonging to different species of *Taenia* (Voge, 1962). The cysticercus consists of a scolex, a neck and a bladder full of fluid. The fluid is probably composed of the metabolic products of the parasite in a transudate of the host's body fluid (Frayha, 1971).

The present investigation is a comparative study of the lipid composition of both fluid and tissue of *T. hydatigena* cysticerci.

**MATERIALS AND METHODS**

*Parasite material*

*Taenia hydatigena* cysticerci were collected from the omentum of 6-12-month-old Syrian sheep, *Ovis aries* var. *Crassicanthus*, within a few hours of being slaughtered in local abattoirs. The cysticerci were freed by blunt dissection from the host membranes and the fluid was collected in a beaker after incising the bladder wall. The fluid was dried by lyophilization in a Virtis lyophilizer (Gardiner, N.Y.). The cysticerci tissues were washed several times with physiological saline and then blotted dry on No. 1 Whatman filter paper. They were divided into two fractions which were weighed separately. One fraction was lyophilized and weighed. The second fraction was used fresh for subsequent determinations.

*Chemicals*

The lipid standards monoolein, diolein, cholesterol palmitate, tripalmitin, lecithin (phosphatidyl choline), cephalin (phosphatidyl ethanolamine), sphingomyelin (phosphatidyl sphingoside), palmitic, heptadecanoic and nonadecanoic acids were purchased from Fluka AG, Buch SG, Switzerland. Cholesterol, lysolecithin (lyso phosphatidyl choline), phosphatidyl inositol and phosphatidyl serine were obtained from Nutritional Biochemical Corporation, Cleveland, Ohio. The fatty acid methylesters, lauric, myristic, palmitic, stearic, arachidic, palmitoleic, oleic, linoleic, linolenic and arachidonic were procured from Mann Research Laboratories, Inc., New York, N.Y. Tridecanoic, myristoleic, pentadecanoic, 11-eicosenoic and 11-14-eicosadienoic acids were supplied by Applied Scientific Laboratories Inc., State College, Pennsylvania. Silica gel-G and Neatan for TLC were products of Fluka AG and of Brinkmann Instruments Inc., Westbury, N.Y., respectively. Columns for GLC were obtained from Varian Aerograph, Walnut Creek, California.

All other chemicals used were reagent grades. All solvent mixtures were made on a v/v basis.
Isolation of lipids

The total lipids of the lyophilized fluid, and of the lyophilized cysticerci were extracted separately according to the method of Folch et al. (1957). Similarly, after cutting the fresh cysticerci into small pieces, their total lipids were extracted by the same method. Two to 5 mg of total lipids from each sample were dissolved in 0.5-1 ml chloroform-methanol (2:1) and chromatographed on TLC plates (Freeman & West, 1966) for the separation of the different classes of lipids, namely phospholipids, monoglycerides, fatty acids, cholesterol, 1,2-diglycerides, 1,3-diglycerides, triglycerides and cholesterol esters. Each class was eluted from the TLC plate and quantitatively determined by the spectrophotometric method of Amenta (1964). Phospholipids were separated from total lipid mixtures on TLC and then fractionated into different components by the method of Parker & Paterson (1965). Each phospholipid component was then analyzed spectrophotometrically for quantitative estimation (Amenta, 1964). The free fatty acid bands eluted from the TLC were methyl esterified according to the method of Metcalf et al. (1966). The bound fatty acids in the mono-, di- and triglycerides were hydrolyzed from each glyceride respectively after elution from TLC, by the technique of Oser (1965). The liberated fatty acids from each glyceride were then chromatographed on TLC, eluted and methyl esterified as described above. The fatty acid methyl esters were chromatographed on two GLC stainless steel columns (10 ft long × 1.8 in. i.d.) in a Varian Aerograph Model 600 D equipped with a hydrogen flame ionization detector. The first column contained 20% butanediol succinate polyester coated on 60-80 Chrom W and was operated at 210°C with a nitrogen flow of 25 ml/min. The second column was comprised of 10% FFAP (an ester of Carbowax 20M with tetraphthalic acid) on a 100-120 Varoport 30 and operated at 190°C with a nitrogen flow of 20 ml/min. Since both GLC systems gave similar results, all the quantitative estimations were determined from the first GLC column.

Placement and recovery studies with reference standards of phospholipids, monoglycerides, diglycerides, triglycerides, fatty acids, cholesterol and cholesterol esters were made, to assist in identifying and quantitatively analysing the lipids of parasitic tissues by means of TLC and GLC as well as to ascertain the efficiency of each step in the chromatographic and spectrophotometric procedures.

RESULTS

The average weights of the total lipids in the tissue and fluid of T. hydatigena cysticerci were respectively 182.22 and 46.65 mg/g of dry weight. One g of lyophilized tissue and 1 g of lyophilized fluid were equivalent to 6.99 g of wet tissue and 74.01 g of fluid respectively. Both tissue and fluid of cysticerci showed the existence of all classes of lipids (Table 1). The phospholipids, triglycerides and cholesterol constituted together about 75 per cent of the total lipids of both tissue and fluid of cysticerci. The remaining lipids namely mono- and diglycerides, fatty acids and cholesterol esters were made to assist in identifying and quantitatively analysing the lipids of parasitic tissues by means of TLC and GLC as well as to ascertain the efficiency of each step in the chromatographic and spectrophotometric procedures.
### Table 1—Quantitative Estimation of the Major Lipid Classes in the Tissue (T) and Fluid (F) of *T. hydatigena* Cysticerci

<table>
<thead>
<tr>
<th>Percentage (w/w) of total lipids</th>
<th>Phospholipids</th>
<th>Monoglycerides</th>
<th>Fatty acids</th>
<th>Cholesterol</th>
<th>Diglycerides</th>
<th>Triglycerides</th>
<th>Cholesterol esters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>F</td>
<td>T</td>
<td>F</td>
<td>T</td>
<td>F</td>
<td>T</td>
</tr>
<tr>
<td><strong>Range of three experiments</strong></td>
<td>34.8</td>
<td>38.1</td>
<td>3.7</td>
<td>1.1</td>
<td>3.5</td>
<td>4.6</td>
<td>9.9</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>41</td>
<td>40</td>
<td>4.9</td>
<td>2.4</td>
<td>6.7</td>
<td>7.0</td>
<td>12.4</td>
</tr>
</tbody>
</table>

### Table 2—Determination of Phospholipids in the Tissue (T) and Fluid (F) of *T. hydatigena* Cysticerci

<table>
<thead>
<tr>
<th>Percentage (w/w) of total phospholipids</th>
<th>Lysolecithin</th>
<th>Sphingomyelin</th>
<th>Lecithin</th>
<th>Phosphatidyl inositol</th>
<th>Phosphatidyl serine</th>
<th>Cephalin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>F</td>
<td>T</td>
<td>F</td>
<td>T</td>
<td>F</td>
</tr>
<tr>
<td><strong>Range of three experiments</strong></td>
<td>14.7</td>
<td>14.4</td>
<td>5.6</td>
<td>5.6</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>15.9</td>
<td>18.3</td>
<td>7.5</td>
<td>9.1</td>
<td>8.5</td>
<td>8.2</td>
</tr>
</tbody>
</table>
Similarly the fatty acids of the mono-, di- and triglycerides showed no differences in concentrations except in C\textsubscript{12:0}, C\textsubscript{14:0}, C\textsubscript{14:1}, C\textsubscript{16:0}, C\textsubscript{18:0} and C\textsubscript{20:2} (Table 3). Only C\textsubscript{20:2} was missing in the triglycerides of the tissue fraction of the cysticerci but highly present in the fluid (Table 3).

**TABLE 3—FATTY ACID (F.A.) COMPOSITION OF THE TISSUE (T) AND FLUID (F) OF T. hydatigena CYSTICERCI**

<table>
<thead>
<tr>
<th>F.A. No. of carbons: No. of double bonds</th>
<th>Methyl esters as percentage (w/w) of total methyl esters</th>
<th>Free F.A.</th>
<th>F.A. of Monoglycerides</th>
<th>F.A. of Diglycerides</th>
<th>F.A. of Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T F</td>
<td>T F</td>
<td>T F</td>
<td>T F</td>
<td></td>
</tr>
<tr>
<td>13:0</td>
<td>1:85</td>
<td>4:30</td>
<td>5:17</td>
<td>5:96</td>
<td>5:01</td>
</tr>
<tr>
<td>14:0</td>
<td>5:03</td>
<td>2:20</td>
<td>2:70</td>
<td>2:06</td>
<td>2:51</td>
</tr>
<tr>
<td>15:0</td>
<td>0:71</td>
<td>0:78</td>
<td>1:12</td>
<td>1:38</td>
<td>1:11</td>
</tr>
<tr>
<td>17:0</td>
<td>0:24</td>
<td>0:19</td>
<td>0:23</td>
<td>0:48</td>
<td>0:28</td>
</tr>
<tr>
<td>18:3</td>
<td>1:14</td>
<td>1:81</td>
<td>2:02</td>
<td>2:06</td>
<td>3:90</td>
</tr>
<tr>
<td>19:0</td>
<td>1:61</td>
<td>1:76</td>
<td>0:00</td>
<td>0:00</td>
<td>0:00</td>
</tr>
<tr>
<td>20:0</td>
<td>0:62</td>
<td>0:78</td>
<td>0:00</td>
<td>0:00</td>
<td>1:39</td>
</tr>
<tr>
<td>20:1</td>
<td>2:99</td>
<td>3:81</td>
<td>2:25</td>
<td>0:00</td>
<td>0:00</td>
</tr>
</tbody>
</table>

It is apparent that the most prevalent free fatty acids as well as those bound to mono-, di- and triglycerides are the even-chain and saturated acids, of which the most common in all determinations were C\textsubscript{16:0} and C\textsubscript{18:0} acids. However, a significantly high degree of unsaturation was also observed in C\textsubscript{14:1}, C\textsubscript{16:1}, C\textsubscript{18:1}, C\textsubscript{20:1} and particularly C\textsubscript{20:2} acids, in all fractions examined (Table 3).

**DISCUSSION**

The total lipids in the tissue of *T. hydatigena* cysticerci (18.2 per cent dry weight) was approximately equal to those reported in *Diphyllobothrium latum* and *Spirometra mansonioides* (17.7 and 16.0 per cent respectively) by Smordintsev & Bebeshin (1936), Totterman & Kirk (1939) and Meyer *et al.* (1966). It fell, however, between those of *M. expansa* and *T. saginata* (30.1 and 31.1 per cent respectively) (Von Brand, 1933; Čmelik & Bartl, 1956) and those of *E. granulosus* cyst membranes and *Taenia taeniaeformis* cysticerci (1.3 and 6.9 per cent respectively) (Čmelik, 1952a; von Brand & Bowman, 1961; McMahon, 1961). It is thus apparent from the little information available on the total lipid composition of cestodes that pronounced differences in concentrations do occur and that particular
trends could not be discernible. The relatively low amount of total lipids in the fluid of *T. hydatigena* cysticerci (4-7 per cent dry weight or 0-063 per cent wet weight) is about five times less than the concentration of total lipids in the blood of sheep which is the host of the parasite. This is probably due to the fact that the fluid in the cysticercus is composed of metabolic products of the parasite diluted in a transudate of the host body fluid (Frayha, 1971).

The ratio of neutral to polar lipids in the cysticerci of *T. hydatigena* is 2 : 1. Although this ratio is not quantitatively similar to that found by Ginger & Fairbairn (1966a) in another cestode *H. diminuta* (3 : 1), the distribution of the components of the neutral lipids are however similar in both parasites with triglycerides being the most abundant (Table 1) followed by cholesterol, cholesterol esters, free fatty acids, di- and monoglycerides. Cholesterol which was reported by Frayha (1971) to be the major component of the unsaponifiable lipids in the scolices of *E. granulosus* and *E. multilocularis* and in the cysticerci of *T. hydatigena*, was demonstrated in this study to be the only sterol detected in *T. hydatigena* cysticerci. It constituted 2-2.5 per cent of the dry weight of the cysticerci of *T. hydatigena*. This value is close to those of *T. taeniaeformis* adults (von Brand et al., 1965) and *E. granulosus* scolices (Konyalian, 1967) which are 1-4 and 3-0 per cent of the dry weight respectively. Only one-third of the cholesterol was in the esterified form in both tissue and fluid of the cysticerci (Table 1).

The phospholipids detected in *T. hydatigena* cysticerci, namely lecithin, cephalin, phosphatidyl inositol, phosphatidyl serine, sphingomyelin and lyssolecithin (Table 2) were the same phospholipids that were reported by Fairbairn et al. (1961) and Ginger & Fairbairn (1966a) in *H. diminuta*. They differed, however, from those of *T. saginata* in that the latter contained no cephalin (Čmelik & Bartl, 1956) and from *T. taeniaeformis* which contained no phosphatidyl inositol (Thompson et al., 1960). Phosphatidyl inositol displayed the highest value among the phospholipids of both tissue and fluid of *T. hydatigena* cysticerci (Table 2), a fact not observed in any other cestode so far studied. The ratio of lecithin to cephalin in the fluid fraction of *T. hydatigena* cysticerci was 1 : 1 which is similar to that found in *T. taeniaeformis* larvae (McMahon, 1961). On the other hand, this ratio in the tissue of the cysticerci fell below one. This is not observed in other cestodes which usually exhibited ratios of 1 or above (McMahon, 1961; Harrington, 1965).

It was noticed from the results (Table 3) that most of the identified fatty acids were of even carbon number and carbon chain length less than 20. For example the most common free as well as bound fatty acids in the tissue and fluid of *T. hydatigena* cysticerci were C_{14}:0, C_{14}:1, C_{16}:0, C_{16}:1, C_{18}:2, C_{20}:0, C_{20}:1 and C_{20}:2. They constituted above 90 per cent of the total identified fatty acids in all determinations. The saturated as well as the unsaturated C-18 (all 18-carbon chain) fatty acids displayed the highest percentage among all fatty acids in both tissue and fluid. For example all 18-carbon free fatty acids of the tissue represent 41-5 per cent of total free fatty acids (27-6 per cent in the fluid). This observation is to a certain extent comparable to that reported by Ginger & Fairbairn (1966a) on *H. diminuta* in which the C-18 acids were 44-2 per cent of the total acids.
Of the saturated free as well as bound fatty acids of the cysticerci stearic (C₁₈:₀) and palmitic (C₁₆:₀) acids were the most abundant. This observation is in agreement with the fact that these two acids were the most common saturated fatty acids in animal lipids (White et al., 1964). However, none of the other predominant fatty acids in animal lipids, namely oleic (C₁₈:₁) palmitoleic (C₁₆:₁) which have been reported to be present in the triglycerides of certain tapeworms (Bailey & Fairbairn, 1968) were observed in T. hydatigena cysticerci. Only linoleic acid (C₁₈:₂) was present in trace amounts (Table 3). It is speculated that in T. hydatigena cysticerci stearic and palmitic acids although highly excreted into the fluid, are still the most important acids retained in the tissue, probably to be used for glycerides metabolism and particularly the triglycerides in which C₁₈:₀ alone had reached 61.82 per cent (C₁₀:₀, 18.76 per cent) of all fatty acids in the tissue fraction (Table 3). This is about fifteen- to twenty-fold more than that determined in the triglycerides of H. diminuta (Ginger & Fairbairn, 1966a).

It was also observed that about 25 per cent of the total identified free fatty acids in the tissue fraction of the cysticerci and 44 per cent of those in the fluid fraction were unsaturated. The unsaturation was observed in the C-14, C-18 and C-20 fatty acids (Table 3). It increased in the fatty acids of the mono- and diglycerides fractions (about 38 and 52 per cent in the tissue respectively and 48 and 66 per cent in the fluid respectively). It dropped, however, in the fatty acids of the triglycerides (12 and 30 per cent in tissue and fluid respectively). It is not yet clear how the anaerobic T. hydatigena cysticerci acquired the unsaturation into its fatty acids because it is well established that molecular oxygen is required for the unsaturation (Erwin & Bloch, 1964a, b). It could therefore be postulated that the unsaturated fatty acids existed in T. hydatigena cysticerci not via a de novo synthesis but through chain-lengthening of certain unsaturated fatty acids that were absorbed as such (i.e. unsaturated) from the host. This observation was noticed by Jacobsen & Fairbairn (1967) in H. diminuta. Further studies are needed to clarify this speculation in T. hydatigena cysticerci.

The 11,14-eicosadienoic (C₂₀:₂) acid was only present in high concentrations in the triglycerides of the fluid fraction of the cysticerci (21.6 per cent) and totally absent in the tissue fraction (Table 3). No explanation could be given at this stage for this discrepancy.

Despite the high degree of specialization of T. hydatigena cysticerci for parasitic life, it is clear that their lipids display in general no unique pattern when compared to other cestodes studied. The only remarkable feature was the appreciable degree of unsaturation of its fatty acids. Further investigations are needed to elucidate the cause of this feature.

Acknowledgement—This study was supported in part by Grant No. 18-5511 from the Research Committee of the Faculties of Medical Sciences, American University of Beirut.

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LIPIDS OF TAENIA HYDATIGENA CYSTICERCII


*Key Word Index*—Lipids; cysticerci; *Taenia hydatigena*; phospholipids.