

# THE LIPID COMPOSITION OF *ASPIDOGASTER CONCHICOLA* VON BAER, 1826

Horace H. Bailey and Charles O. Rock<sup>1</sup>

Department of Biology, West Texas State University, Canyon, Texas

The major lipid classes and their constituent fatty acids in *Aspidogaster conchicola* (Trematoda) were determined by a combination of thin-layer and gas-liquid chromatography. The fatty acid composition is characterized by high levels of 16- and 18-carbon fatty acids. The predominate saturated acids being palmitic (21.5%) and stearic (25.6%), with the major unsaturated acids being linolenic (11.1%) and arachidonic (11.0%). Sterols were the primary neutral lipid present and were comprised chiefly of cholesterol (92%). Hydrocarbons were also found to occur in the neutral lipids but preliminary evidence indicates these hydrocarbons were most likely derived from oil pollution of the stream and were not products of either the host or parasite. Phosphatidylcholine was the major phospholipid component. Lesser amounts of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol also were detected.

The Aspidogastriid trematodes are parasites primarily of poikilothermic animals, chiefly molluscs and crustaceans. Presently little information is available concerning the chemical composition and metabolism of these worms, even though certain species are amenable to this type of study, i.e., they can be maintained *in vitro* for relatively long periods of time.

Recently, we reported on the ultrastructure of the integument (1) and on the histochemical distribution of certain enzyme systems (2, 3) in one member of this group, *Aspidogaster conchicola*, which occurs as a symbiont in the pericardial and renal cavities of many species of fresh water mussels. In this paper we report on the lipid composition of *A. conchicola*, data which are a necessary prerequisite to future studies concerning lipid metabolism within the parasite-host relationship.

## MATERIALS AND METHODS

Unionid collections were made from Medicine Creek, 0.4 km south of Medicine Park, Comanche County, Oklahoma. In all cases, a single species of mussel, *Anodonta grandis*, served as a source of *A. conchicola*. Worms were rinsed from the pericardial cavity with mussel Ringer's solution, blotted dry, and wet weights determined.

## Lipid extraction and purification

Total lipids were extracted into 20 volumes of chloroform:methanol (2/1) (4) and purified by passage through Sephadex columns (5). Solvents were reagent grade, but purified, nevertheless, by accepted procedures before use (6). They were prepared on a volume basis and contained 2,6-di-*tert*-butyl-*p*-cresol in a concentration of 50 mg/l as an antioxidant (7).

## Separation and identification of lipids

After purification total lipid samples were applied to 20 x 20 cm glass plates coated with a 0.5 mm layer of Silica Gel G, and the neutral lipids fractionated by unidimensional double development (8). The first solvent (diethyl ether:benzene:ethanol:acetic acid, 40/50/2/0.2) was allowed to advance 16 cm from the origin, and the second (hexane:diethyl ether, 94/6), to the top of the plate. Another solvent system, hexane:diethyl ether:acetic acid (80/20/1) also was used. Neutral lipid components were identified by comparison with standards and analysis of degradation products.

Polar lipids were resolved on Silica Gel G layers developed in chloroform:methanol:water (65/25/4) or on Silica Gel H layers (9). Polar lipids were identified by comparison with standards run on the same plate and by specific spray reagents.

In addition, plates were either charred as an aid in determining the lipid classes or visualized non-destructively with the aid

<sup>1</sup> Present address: Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830

of Rhodamine 6 G or 2,7-dichlorofluorescein and UV light.

#### Gas-Liquid Chromatography

Methyl esters were prepared by treating specific lipid classes isolated after thin-layer chromatography (TLC) with anhydrous HCl-methanol in a sealed tube under nitrogen overnight at room temperature. HCl-methanol was generated within 2 weeks of use and stored at 4 C to diminish the formation of methyl chloride, water (10, 11) and other by-products (12) which decrease yields. Methyl esters were extracted into hexane and purified by TLC on Silica Gel G layers developed in hexane: diethyl ether:acetic acid (80/20/1). The methyl ester band was eluted with spectral grade hexane and injected into the gas chromatograph in carbon disulfide.

Methyl esters were identified by comparison with standards, relative retention times (13) and equivalent carbon numbers (14). To aid in the identification of unsaturated components, their methyl esters were fractionated according to degree of unsaturation on Silica Gel G layers impregnated with 5% (w/w) silver nitrate and developed in reagent grade chloroform. After separation gas chromatograms of these unsaturated methyl esters were compared both before and after hydrogenation (15).

Most analyses were carried out on a Varian Series 1700 gas chromatograph equipped with a hydrogen flame detector. Test procedures of Horning *et al.* (16) were used to calibrate the instrument. Quantitative results with National Heart Institute Fatty Acid Standards (A, B, and D) agreed with the stated composition data with a relative error of less than 2% for major components (>10% of total mixture) and less than 3% for minor components (<10% of total mixture).

For fatty acid identification, a column 6 feet long and 2 mm in diameter packed with Gas Chrom Q (100 to 120 mesh) coated with 1.5% diethyleneglycol succinate was used with the chromatograph operating isothermally at 180 C.

The sterols isolated by TLC were subjected to gas-liquid chromatography in their free form or as acetates at 205 C on Gas Chrom Q (100 to 120 mesh) carrying 3% QF-1.

Hydrocarbons were isolated in one of the neutral lipid systems noted previously and purified from any contaminating sterol esters by rechromatography on Silica Gel G layers developed in hexane. Tentative identification of hydrocarbons was obtained by gas-liquid chromatography using a 6 ft. column packed with Gas Chrom Q (100 to 120 mesh) carrying 1% OV-1 and linearly programmed from 150 to 200 C at 4 C/min. In addition, hydrocarbons also were chromatographed on a Hewlett Packard Series 500 gas-chromatograph equipped with a 300 ft. capillary column coated with 0.03% Igepal Co-990 and linearly programmed from 110 to 180 C at 40 C/min.

#### RESULTS AND DISCUSSION

*A. conchicola* contains 2.8% lipid on a wet weight basis, with neutral lipids comprising 63.4% of the total, and phospholipids 44.6%. Thin-layer chromatographic separations of *A. conchicola* neutral lipids indicate that the major component was a sterol fraction with lesser amounts of triglycerides, free fatty acids, sterol esters, and hydrocarbons. The phospholipid fraction was dominated by phosphatidylcholine with smaller amounts of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. There were little or no lysophosphatides or other phospholipids such as sphingomyelin. This distribution of lipid classes is quite similar to that which has been reported for the adult human blood fluke, *Schistosoma mansoni*. In that species sterols constituted approximately half of the neutral lipid (17), with the phospholipids comprising 37% of the total. On the other hand, the major neutral lipid component in cestodes (18, 19) and acanthocephalans (20) was triglycerides.

The major fatty acids occurring in the total lipids of *A. conchicola* are presented in Table 1. High levels of C<sub>18</sub> (52.6%) and C<sub>16</sub> (25.8%) fatty acids were found as well as a relatively high percentage of unsaturated components (42.3%). In contrast to the similarities noted previously in regard to lipid class composition this distribution of fatty acids is quite distinct from that known to occur in *S. mansoni* (21). This is not particularly surprising in the light of the lack of a capacity for *de novo* synthesis of fatty acids in many free living and parasitic species of the phylum *Platy-*

TABLE 1. *Component fatty acids in the total lipids of A. conchicola*

Fatty Acid <sup>a</sup>	Mole % <sup>b</sup>
14:0	6.7
15:0	1.0
16:0	21.5
16:1	4.3
17:0	+ <sup>c</sup>
18:0	25.6
18:1	7.9
18:2	8.0
18:3	11.1
20:4	11.0

<sup>a</sup> Number of carbons; number of double bonds.

<sup>b</sup> Mean of six determinations from two separate lipid samples.

<sup>c</sup> Trace amounts (less than 1%).

*helminthes* (22), and the demonstration that in some members of this phylum the fatty acid constituents closely resemble those of the host organism (23).

The fatty acid composition of each neutral lipid class in *A. conchicola* is outlined in Table 2. The triglycerides were characterized by high titers of arachidonic and palmitic acids. Again these data are in sharp contrast to those of *S. mansoni* in which oleic acid was the predominate fatty acid esterified to glycerol (21), and the cestode *Hymenolepis diminuta* in which linoleic acid was the major fatty acid component of the triglycerides (24). The free fatty acid pool of *A. conchicola* was typified by high levels of saturated fatty acids (79%), with palmitic (47.8%) and stearic (23.1%) being the major constituents. The major acid esterified to *A. conchicola* sterol was oleic (73.3%) but considerable quantities of palmitic acid (16.9%) also were detected.

TABLE 2. *Fatty acid composition of the neutral lipids of A. conchicola*

Fatty Acid	Triglyceride	Free Fatty Acid	Sterol ester
14:0	12.2 <sup>b</sup>	7.1	2.4
15:0	+ <sup>c</sup>	1.0	+
16:0	24.5	47.8	16.9
16:1	6.4	1.5	1.0
18:0	9.1	23.1	5.5
18:1	9.1	11.6	73.3
18:2	8.9	7.9	1.0
18:3	8.6	—	—
20:4	20.8	—	—

<sup>a</sup> Number of carbons; number of double bonds.

<sup>b</sup> Mean of six determinations from two separate lipid samples in mole %.

<sup>c</sup> Trace amounts (less than 1%).

The primary sterol of *A. conchicola* as revealed by gas-liquid chromatography was cholesterol (92%), (Table 3) but the presence of campesterol was also noted (8%). Other studies on sterol composition in trematodes have shown that the non-saponifiable fractions of adult *Gastrothylax crumenifer* (25) and *S. mansoni* (17) consist mainly of cholesterol along with minor amounts of a few closely related sterols. It is speculated that the sterols of *A. conchicola* are derived from the environment, since studies on the lipid metabolism of flatworms demonstrated that some of these organisms do not possess the oxygen-dependent pathways necessary for synthesis of sterols (26, 27).

TABLE 3. *Sterols of A. conchicola*

Sterol <sup>a</sup>	Mole % <sup>b</sup>
Cholesterol	92.0
Campesterol	8.0

<sup>a</sup> Cholesterol; 5-cholesten-3 $\beta$ -ol; campesterol; 24 $\beta$ -methyl-5-cholesten-3 $\beta$ -ol

<sup>b</sup> Mean of six determinations from two separate lipid samples.

The outstanding feature of the hydrocarbon spectrum was that the components did not resemble a natural assemblage of chain lengths and branching patterns normally associated with biological systems. The major peaks within the complex mixture were C<sub>12</sub> and C<sub>26</sub>, although it should be noted that the methods used to prepare the samples precluded the detection of any short-chained components even if they were originally present. The atypical nature of the hydrocarbon spectrum points to a source of these compounds other than biological synthesis. Fresh-water clams have been utilized as indicators of water pollution and it is not unlikely that the parasite hydrocarbons are derived from the mollusc, which in turn has concentrated these organics from the water during the course of feeding. In support of this hypothesis, hexane extracts of water samples taken from the collecting site contain a relatively high concentration of these types of hydrocarbons. Additionally, preliminary experiments designed to determine the extent of incorporation of labeled acetate, glycerol, glucose, palmitate, and oleate into the lipids of *A. conchicola* failed to show any labeling of the hydrocarbon fraction, although sev-

eral other lipid components were rapidly labeled.

The fatty acids present in the various phospholipid classes of *A. concibicola* are summarized in Table 4. In general, the pattern of structural organization of the phospholipid classes is similar to those found in many other tissues. The major saturated acid in phosphatidylcholine is palmitic, whereas in phosphatidylinositol and -serine, stearic acid predominates. On the other hand phosphatidyl ethanolamine contains essentially equal amounts of palmitic, stearic, and oleic acids.

TABLE 4. Component fatty acids of the phospholipids of *A. Concibicola*

Fatty Acid	Phosphatidylcholine	Phosphatidylserine + Inositol	Phosphatidylethanolamine
14:0	6.2 <sup>b</sup>	.6	2.9
15:0	+ <sup>c</sup>	+	+
16:0	42.5	17.1	23.5
16:1	4.9	4.3	4.5
18:0	15.1	29.2	24.7
18:1	18.2	22.3	24.6
18:2	2.0	4.9	5.0
18:3	10.4	7.6	8.7
20:4	+	11.0	5.7

<sup>a</sup> Number of carbons; number of double bonds.

<sup>b</sup> Mean of six determinations from two separate lipid samples in mole %.

<sup>c</sup> Trace amounts (less than 1%).

The pathways of lipid synthesis and metabolic regulation which are used to produce the lipid composition and fatty acid distribution noted in *A. concibicola* are not known. Experiments designed to elucidate the means of procurement and metabolism of lipids in this species are currently being conducted.

#### ACKNOWLEDGMENTS

The authors express their appreciation to Dr. Loren G. Hill, University of Oklahoma Biological Station, and Dr. Paul G. Risser, Oklahoma Biological Survey, for providing facilities and financial support during the course of this study. This investigation was supported in part by U. S. Public Health

Service Grant AI 08971 from the National Institute of Allergy and Infectious Diseases.

#### REFERENCES

- H. H. BAILEY and S. J. TOMPKINS, *J. Parasit.* 57: 848-854 (1971).
- J. J. TRIMBLE, H. H. BAILEY and E. N. NELSON, *Expl. Parasit.* 29: 457-462 (1971).
- J. J. TRIMBLE, H. H. BAILEY and A. SHEPARD, *Expl. Parasit.* 32: 181-190 (1972).
- S. POLCH, M. LEES and G. H. SLONE-STANLEY, *J. Biol. Chem.* 226: 497-509 (1957).
- A. N. SIAKOTOS and G. ROUSER, *J. Am. Oil Chem. Soc.* 42: 913-919 (1965).
- A. I. VOGEL, *Practical Organic Chemistry*, Longmans, London, 1957.
- J. J. WREN and A. D. SZCZEPANOWSKA, *J. Chromatog.* 14: 405-410 (1964).
- C. P. FREEMAN and D. WEST, *J. Lipid Res.* 7: 324-327 (1966).
- V. P. SKIPSKI, R. F. PETERSON and M. BARCLAY, *Biochem. J.* 90: 374-378 (1964).
- S. R. CARTER and J. A. V. BUTLER, *J. Chem. Soc.* 125: 963 (1924).
- Y. KISHIMOTO and N. S. RADIN, *J. Lipid Res.* 6: 435-436 (1965).
- P. V. JOHNSTON and B. I. ROOTS, *J. Lipid Res.* 5: 477-478 (1964).
- R. G. ACKMAN and R. D. BURGER, *J. Am. Oil Chem. Soc.* 42: 38-42 (1965).
- H. H. HOFSTETTER, N. SEN and R. T. HOLMAN, *J. Am. Oil Chem. Soc.* 42: 20-27 (1965).
- J. W. FARQUHAR, W. INSULL, P. ROSEN, W. STOFFEL and E. H. AHRENS, *Nutrit. Rev.* 17(8), Part II Supplement (1959).
- R. A. HORNING, E. H. AHRENS, S. R. LIPSKY, F. H. MATTSON, J. E. MEAD, D. A. TURNER and W. H. GOLDWATER, *J. Lipid Res.* 5: 20-27 (1964).
- T. M. SMITH and T. J. BROOKS, *Parasit.* 59: 293-298 (1969).
- C. D. GINGER and D. FAIRBAIRN, *J. Parasit.* 52: 1086-1096 (1966).
- F. MEYER, S. KIMURA and J. F. MUELLER, *J. Biol. Chem.* 241: 4224-4232 (1966).
- C. G. BEAMES and F. M. FISHER, *Comp. Biochem. Physiol.* 13: 401-412 (1964).
- T. M. SMITH, T. J. BROOKS and H. B. WHITE, *Lipids* 4: 31-36 (1969).
- D. FAIRBAIRN, *Biol. Rev.* 45: 29-72 (1970).
- G. H. BUTEAU, J. E. SIMONS and D. FAIRBAIRN, *Expl. Parasit.* 26: 209-213 (1969).
- G. H. BUTEAU and D. FAIRBAIRN, *Expl. Parasit.* 25: 265-275 (1969).
- M. M. GOIL, *Parasit.* 54: 81-85 (1964).
- G. F. FRAYHA and D. FAIRBAIRN, *Comp. Biochem. Physiol.* 28: 1115-1125 (1969).
- T. M. SMITH, T. J. BROOKS and V. G. LOCKARD, *Lipids* 5: 854-856 (1970).