# STUDIES ON THE LIPID METABOLISM OF THE METACERCARIAE OF CLINOSTOMUM COMPLANATUM

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT
FOR THE DEGREE OF
MASTER OF PHILOSOPHY

By
JAWED SIDDIQUI

SECTION OF PARASITOLOGY
DEPARTMENT OF ZOOLOGY
ALIGARH MUSLIM UNIVERSITY
ALIGARH

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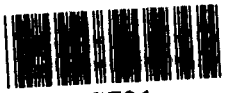

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19 February 1981

This is to certify that the work presented in this dissertation has been carried out by Jawed Siddiqui and is suitable for the award of the Master of Philosophy degree in Zoology of the Aligarh Muslim University, Aligarh.

ATHER H. SIDDIQI
Professor of Parasitology

DEDICATED

TO

MY FATHER

IN

GRATITUDE

# C O N T E N T S

# LIST OF TABLES AND FIGURE

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I express my gratitude to Prof Ather H. Siddiqi under whose supervision and guidance this research work was carried out.

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JAWED SIDDIQUI

# C H A P T E R - I

## INTRODUCTION

The damaging effect of the metacercariae (the temporary parasites in the sense that they are on route to another host, in many cases the definitive host) have upon their host is largely dependent on whether or not they encyst. Some metacercariae do not encyst within the second intermediate hosts and continue in an active state, feeding on tissues of their host and thus prove to be pathogenic. The study of such metacercariae is important not only because they are pathogenic in their transitional stage and lead a parasitic way of life in aquatic animals (which are closely linked to the feeding pattern of the final hosts) but also because in doing so they cause enormous losses to our edible aquatic fauna, mostly fishes, before being transmitted to the final host. There are numerous reports on the occurrence and pathogenecity of metacercariae in or on the edible aquatic fauna mostly fishes and so it is not possible to enlist them. The clinostomatid metacercariae alone have been reported from various species of fishes and are known as "yellow grub".

Studies on the physiological aspects of trematodes in general and lipid metabolism in particular have made a late begining due to the fact that the modern techniques

like column, thin layer and gas liquid chromatography for the identification of lipids were applied to these parasites only during the last one decade. The literature available on the physiology of parasites in general and metacercariae in particular concerning lipid distribution, leakage, utilization, synthesis etc., is sparse. (This may be due to the infrequent availibilty of the metacercariae as they inhabit poorly understood habitats. They are difficult to culture in vitro , and comprise a challenging group of organisms to work with). Though morphological and taxonomical studies on metacercariae have been a popular branch of research with the Indian parasitologists, a great deal remains to be done in relation to the physiological and biochemical aspects of the metacercariae.

# C H A P T E R - II

## HISTORICAL REVIEW

The lipid metabolism of trematodes has remained more or less a neglected subject. Whatever little work has been done, concerns mainly a few adult trematodes (von Brand, 1973 ; Fried & Pucci, 1976 ; Yusufi & Siddiqi, 1976 ; Fried & Appel, 1977 ; Yusufi & Siddiqi, 1977 ; Fried & Butler, 1977 ; Fried & Boddorff, 1978 ; Fried & Shapiro, 1979) and relatively less work has been done on the larval trematodes. The lipids are known to be present as reserve food in miracidia although not in as large a quantity as glycogen (Axmann, 1947 ; von Brand, 1973). No information is yet available whether the stored lipids are employed as energy source during larval stages. Ginetsinskaia ( 96), who studied the lipid composition in eight species of trematodes, reported that the amount of lipids present in sporocyst walls was directly correlated with the sites which they occupied within the molluscan host. Those located on the surface of the host's intestine were completely devoid of lipids as were those situated between organs; however, the sporocyst of Cotylurus brevis and Cercaria spinulosa , which were embedded in the host's hepato-pancreas, were rich in lipid content. Cheng & Snyder (1962) have also found fatty acids adhering to, and

the interior of, the sporocyst wall of Glypthelmins pennsylvaniensis. James & Bowers (1967) have reported lipids in the daughter sporocyst of Cercaria bucephalopsis. Pascoe & Richards (1970) have observed lipids and lipid oxidation in sporocysts of Cercaria dichotoma. Recently, phospholipids and neutral lipids have been reported from the daughter sporocysts of Microphalus similis (McManus et al., 1975). There are only two evidences of the presence of lipids in rediae: one is that reported by Ginetsinskaia (1961) in the rediae of members of the Echinostomatidae and Notocotylidae which were embedded in the hepato-pancreas of their molluscan hosts and the other is by Hoskin, Cheng & Shapiro (1974) who have shown sterols, free fatty acids, triglycerides and sterol ester in the rediae of Himasthala sp. The lipids are present in the bodies of certain cercariae where they are limited to the excretory system and to the parenchyma (Lutta, 1939; Ginetsinkaia & Dobrovolskii, 1962 ; Cheng, 1965). Cheng & Snyder (1962) by employing histochemical techniques, pointed out that the fats found in the body parenchyma of Glypthelmins pennsylvaniensis cercariae were not neutral fats but unsaturated fatty acids. Similar reports have also been given by Faust & Tallqvist (1907), von Brand (1928) and Smorodintsev & Bobeshin (1939) from three flat-worms, Dibothriocephalus latus, F. hepatica and Taeniarhynchus saginatus respectively. The cercaria of S. mansoni contains about 19% dry weight lipid according to Kent (1963) and include seven lipid classes consisting of 26 fatty acids according to Smith et al. (1966).

From the information available, it seems that the presence and maintenance of lipids in metacercariae appear to be influenced by the sites of encystment within the second intermediate host. Ginetsinskaia (1961) reported that in the metacercariae of Sphaerostoma which were embedded between the organs of its second intermediate hosts and those of Opisthioglyphae ranae which were encysted in the foot tissues and in the mantle of Lymnea stagnalis, the stored lipids gradually decreased, completely disappearing in one to two months. On the other hand the amount of stored lipids increased in the metacercariae of Cercaria spinulosa and Cotylurus brevis five days after the cercariae penetrated their intermediate hosts. Lipids have been demonstrated in the tissues of some metacercariae (Ginetsinskaia, 1961; Erasmus & Ohman, 1963; Ždárská, 1964). Erasmus (1967) has localized lipids in the excretory as well as cytoplasmic lining of the reserve bladder system in the metacercariae of Cyathocotyle bushinensis. Recently, accumulation and excretion of neutral lipids have been reported from the metacercariae of Leucochloridiomorpha constantiae (Harris & Cheng, 1973; Fried & Shapiro, 1975), Echinostoma revolutum (Butler & Fried, 1977) and Cotylurus sp., (Fried & Butler, 1977). Although oxidative degradation of fats is a universal phenomenon among all living organisms, as yet no adult parasitic helminth has been shown to possess a complete sequence of $\beta$-oxidation pathway, whereas the developing eggs of Ascaris lumbricoides and free-living larvae and free-living adults of Strongyloides ratti have a functional

β-oxidation sequences (Ward & Fairbairn, 1970; Korting & Fairbairn, 1971).

Lipases have been reported in the intestinal canal of some helminths, e.g., A. lumbricoides, Strongylus edentatus, Leidynema appendiculata (Rogers, 1941; Carpenter, 1952; Lee, 1958) and in the alimentary canal of F. hepatica (Pennoit-De Cooman & van Grembergen, 1942). Tissue lipases of unknown significance, presumably assisting in lipid movement within tissues rather than preparing lipids for absorption from the intestinal tract, have been reported from the tissues of filariform larvae of S. ratti and Nippostrongylus muris and less pronounced in cercariae of S. mansoni (Mandlowitz et al., 1960). The lipase has also been registered from the surface of the developing cercariae of Bucephalus sp., (Cheng, 1965). Recently, lipase has been demonstrated histochemically in various organs of the metacercariae of C. complanatum (Alam & Nizami, personal communication).

Some physiological work has also been done on the metacercariae of C. complanatum e.g., $CO_2$ fixation, incorporation of $^{14}CO_2$ into different amino acids and into intermediates of TCA cycle (Thomas & Gallicchio, 1967a) and incorporation of $^{14}C$-glucose in vivo (Thomas & Gallicchio, 1967b). The clinostomatid metacercariae also produce marked pathogenic effects on their intermediate hosts as reported by Rai (1968) and may even cause sterlity as shown by Nassi (1976). Singh & Virmani (1978) observed

the dislodging of the scales and decrease of haemoglobin content, PCV and increase in erythrocyte sedimentation rate (ESR) in the fishes which were infected with the metacercariae of C. piscidium.

# C H A P T E R - III

## STATEMENT OF PROBLEM

Since very little is known to-date concerning the physiology and biochemistry of lipids in the larval forms, the author chose to study the physiological and biochemical aspects of lipids in the metacercarial form of C. complanatum. It is of very common occurrence in the body cavity of a fresh water fish, Trichogaster fasciatus. The metacercaria is metabolically active (Thomas & Gallicchio, 1967a,b; Siddiqui & Nizami, in press) and morphologically comparable to its adult except that egg production is lacking. Hence, it was decided to determine, whether the transformation of the metacercaria into an adult, which is accompanied by profound physico-chemical changes, results in any change in the lipid biochemistry. Practically, it is either the cercarial or the metacercarial stage which is actively or passively infective, hence, complete information of these larval stages are obviously needed.

An attempt has been made to determine the total lipid content as well as its fractions in the metacercariae of C. complanatum. Similarly, a number of some selected hormones and chemicals were tested for their inhibitory or stimulatory effect on the lipase activity in the metacercariae.

# C H A P T E R - IV

# MATERIALS AND METHODS

(A) Biological

The metacercariae of C. complanatum were obtained from the body cavity of a fresh water fish, Trichogaster fasciatus which were collected from the near-by ponds and ditches of Aligarh and maintained in the laboratory in glass aquaria. The metacercariae were kept in the modified Ringer's saline (Forster & Taggart, 1950, containing NaCl 100mM; KCl 2.5mM; $CaCl_2$ 1.5mM; $MgCl_2$ 1mM; $NaH_2PO_4$ 0.5mM and $NaHCO_3$ 5mM) at room temperature.

(B) Extraction of lipids

Prior to the lipid extraction, the active metacercariae were quickly damp dried on a Whatman filter paper and weighed on a single pan balance. The total lipids were extracted by the method of Folch et al. (1957) as modified by Misra (1968). The metacercariae were transferred to 10-20 volumes of chloroform-methanol in the ratio of 2:1 (v/v) and allowed to stand overnight with occasional shaking at room temperature. They were homogenized in the same solvent and the homogenate was centrifuged. The supernatant was separated and the residue was re-extracted twice with 10 more volumes of chloroform-methanol for two hours

at room temperature and again centrifuged and the supernatants were pooled and evaporated to dryness in vacuum at 40-50°C in a rotary flask evaporator.

The dried residue was dissolved in chloroform-methanol containing 4% distilled water (v/v). It was evaporated to dryness in vacuum at 45-50°C. This step was repeated twice to separate protein-bound lipids. This final dried residue was again dissolved in chloroform-methanol and washed with 0.88% KCl in a separatory funnel, gently mixed and allowed to stand at room temperature until the two phases separated clearly. The lower chloroform-methanol layer was collected and the upper layer was washed twice with chloroform-methanol and the chloroform-methanol layers were pooled and evaporated to dryness in vacuum at the same temperature mentioned earlier. This final dried residue was dissolved in a known volume of pure chloroform for lipid analysis.

(C) Estimation of total lipids

The total lipid of the extracted sample was determined by the method of Zöllner & Krisch (1962). One ml of lipid extract was taken in a test tube and 4 ml of concentrated $H_2SO_4$ was added into it and boiled for 10 min, cooled and shaken. Suitable aliquots were transferred into seven test tubes except one in which equal amount of conc. $H_2SO_4$ was added in place of the aliquot which served as a reagent blank. To each tube including the blank 4 ml of Zöllner

reagent was added (Zöllner reagent was prepared by adding 13mM vanillin in 14mM ortho-phespheric acid) and after seme time the developed color was read against the reagent blank. The total lipids were calculated from a calibration curve previously prepared by using a standard total lipid of known strenght.

(D) Estimation of the fractions of lipids

(i) Total cholesterol

Total cholesterol was estimated by the methods of Zlatkis, Zak & Boyle (1953) and Sackett (1925). In the first method, suitable aliquots were transferred into seven test tubes except one in which equal quantity of chloroform was added in place of aliquot as control, 3 ml of glacial acetic acid was added to each tube including the blank, and mixed thoroughly and then 2 ml of $FeCl_3$ reagent (freshly prepared by taking one ml of 10% $FeCl_3$ (v/v) in glacial acetic acid, diluted to 100 ml with conc. $H_2SO_4$ acid) was carefully added from the side of the test tube to allow the formation of a brown ring. The tubes were shaken thoroughly, cooled and color densities were read against a reagent blank. The total cholesterol was calculated from a calibration curve previously prepared by using a cholesterol solution of known molarity prepared in glacial acetic acid. In the second method, suitable aliquots of lipid extract were transferred into seven test tubes except one in which equal amount of chloroform was added in place of the aliquot as

control. Five ml chloroform was added in each tube and shaken vigorously for some time; later 2 ml of ice-cold acetic anhydride and concentrated $H_2SO_4$ in the ratio of 20:1 was added. This was left in the dark at room temperature (~25°C). After 15 min the developed green color was read against the reagent blank. The total cholesterol was calculated from a calibration curve previously prepared by using a cholesterol solution of known strenght in chloroform.

(ii) Total phospholipids

The total phospholipids were determined by the method of Bartlett (1950) as modified by Marinetti (1962). Suitable aliquots of lipid extract were transferred into seven test tubes except one in which equal amount of chloroform was added in place of equal aliquots. The contents of the tubes were evaporated to dryness and the dry contents of the tubes were digested on an electric digestion unit with 0.8 ml 70% PCA for 25-30 min (2-3 glass beads were added in each tube to avoid bumping). On cooling, 7 ml distilled water was added into each tube followed by 1.5 ml 2.5% ammonium molybdate (w/v). After thorough mixing of the contents, 0.2 ml of α-amino β-hydroxy naphthalene S-sulfonic acid (prepared by adding 2.5 g sodium bi-sulphite, 0.5 g sodium sulphite and 0.042 g ANSA in 250 ml distilled water) was added in each tube. They were then capped and heated in boiling water-bath for exactly seven min, cooled under

tap water and the developped blue color was read against the reagent blank. Total phosphorus was calculated from a calibration curve previously prepared by using a $KH_2PO_4$ solution of known molarity as standard. The phospholipid values were obtained after multiplying the phospholipid phosphorus by a factor of 25 (Subrahmanyam & Venkatesan, 1968).

(iii) Total triglycerides

Total triglycerides were determined by the method of van Handel & Zilversmit (1957). Four g of Zeocarb-225, placed in a glass stoppered 100 ml conical flask, was moistened with 2 ml chloroform. After adding a suitable amount of lipid extract, 10-20 ml chloroform was added. The contents were allowed to stand for one hour at room temperature, with intermittent shaking. They were filtered through a Whatman filter paper No. 1. Suitable aliquots were transferred into seven test tubes except the blank in which equal amount of chloroform was added in place of aliquots and then the solvents were allowed to evaporate. The tubes were kept at 60-70°C for 15 min after adding 0.5 ml of 0.4% alcoholic KOH (w/v) followed by 0.5 ml of 0.2N $H_2SO_4$ acid. Alcohol was removed by immersing the tubes for 15 min in a gently boiling water-bath. After cooling, triglyceride contents were determined by periodate oxidation. To the tubes 0.1 ml of 0.5M sodium periodate solution was added and the oxidation was stopped exactly after 10 min by the addition of 0.1 ml of 0.5M sodium

arsenite. A yellow color of iodine appeared which disappeared within few minutes, then 9 ml ofv0.24% (w/v) chromotropic acid reagent (in $H_2SO_4$ and $H_2O$, 2:1, v/v) was added and heated for exactly 30 min in a boiling water-bath. The developed color was read against a reagent blank. The quantity of triglycerides is represented in terms of weight of mustard oil which has been used to prepare a calibration curve as a standard.

(iv) Free fatty acids

Free fatty acids (FFA) were determined by the method of Itaya & Ui (1965). Suitable aliquots of lipid extract were added to seven stoppered pyrex test tubes, containing 6 ml chloroform and 2 ml of phosphate buffer of pH 7, except blank in which equal amount of chloroform was added in place of aliquot and the tubes were shaken for 90 sec. After a setting period of 15 min, the upper layer was aspirated with PVC capillary, and 3 ml of copper-triethanolamine solution was added (1M triethanolamine : 1N acetic acid : 6.45% $Cu(NO_3)_2$ $3H_2O$, 9:1:10, v/v/v). The tubes were shaken for 10 min and after 15 min the copper-triethanolamine layer was aspirated. The residue chloroform layer was filtered and two drops of 1% sodium diethyl-thiocarbamate solution was added. The yellow brown color, developed immediately, was measured against a reagent blank. Free fatty acids were calculated from a calibration curve prepared by using palmitic acid standard solution in chloroform.

(E) Fractionation and estimation of phopholipids

Phopholipids were further fractionated on scrupulously clean thin layer chromatographic glass-plates of 20x20 cm (Griffin & George, Great Britain) which were coated with a slurry of adsorbent Silica Gel G, containing 13% $CaSO_4$ as binder (from Centron Research Laboratories, Bombay) of uniform layer (about 0.5 mm thick) prepared by vigorously shaking a specific amount of Silica Gel G with approximately a double amount of water in a 250 ml ground glass Erlenmeyer flask. The plates were left to dry at room temperature for 2 hours. They were activated before use in an oven at 110°C for one hour. Definite amount of concentrated lipids were applied on the plates as spots, 3-4 mm in diameter, 2-4 cm from the bottom edge of the plates. The solvent was allowed to evaporate. The chromatoplate was placed in a developing tank of 23x23x7.5 cm (Griffin & George, Great Britain) containing chloroform-methanol-water (65:25:4, v/v/v) as the solvent system, after the method of Skipski, Peterson & Barclay (1964). The chromatogram was developed until the solvent front reached the designated distance from within 10 cm of the origin to the lenght of the plates. The platevwas removed and air dried at room temperature for 20 min . The spots were encircled after visualising them with iodine vapour in another tank. A drop of water was placed on each of the encircled areas, which were removed for analysis by scrapping off with a razor blade. The scrapped Silica Gel was transferred into the centrifuge

tube by a thin knife spatula. The areas in which there were no spots, were also removed to be used as blank. Each spot was eluted from the Silica Gel by suspending the powder in the chloroform and centrifuged. The supernatants were taken and their concentrations, were determined as described aboved. The various fractions were identified by comparing the Rf (Retardation factor) values with standard phospholipids (obtained from V.P. Chest Institute, Delhi) applied on the same plate.

(F) Enzymatic studies

(a) Biological

Prior to the homogenate preparation the active metacercariae were quickly damp dried on a Whatman filter paper and weighed. The homogenate (5-10%, w/v) of the metacercariae was prepared in ice-cold distilled water, using a glass Potter-Elvehjem tissue homogenizer at 4°C. The samples were centrifuged at 1500 x g for 5-10 min to remove cell debris and the supernatant was used for enzyme assay.

(b) Estimation of lipase activity

The lipase (E.C.3.1.1.3) activity was determined colorimetrically by the method of Schmidt, Stork & Dahl (1974) using olive oil as a substrate at pH 8.5. Suitable aliquots of homogenate were transferred in seven test tubes except one (for blank) in which equal amount of chloroform was added. One ml of incubation mixture (olive oil suspension

58mM: sodium deoxycholate 10mM: triethanolamine-HCl buffer 1M, 50:5:45, v/v/v) was added to each tube and incubated at 37± 2°C for 10 min in a water-bath. The reaction was stopped by putting the tubes in boiling water-bath for 1-2 min. Five ml of chloroform followed by 2.5 ml of copper reagent (prepared by dissolving 18.5 g triethanolamine-HCl in 70 ml distilled water and 6.45 g $Cu(NO_3)_2 3H_2O$ in 100 ml distilled water, both the solutions were mixed and pH 7.5 was adjusted with 5N NaOH and diluted to 200 ml with distilled water) were added to the tubes, which were then stoppered and shaken for approximately 20 min and centrifuged. The upper layer was aspirated by polyvinyl chloride capillary (PVC) pump and the lower layer was filtered and 2 ml of filtrates were taken and 0.25 ml color reagent (sodium di-ethyl di-thiocarbamate, 11mM ) was added to each tube and the developed color was read against a reagent blank. Free fatty acids, separated in the form of their copper salts, were calculated from a calibration curve previously prepared by using palmitic acid standard solution in chloroform. The amount of FFAs liberated per unit time under optimal condition is a measure of the lipase activity and the specific activity was expressed as µg FFA liberated per mg protein per hour.

(c) Effect of hormones/chemicals on lipase activity

In order to examine the effect of different kinds of hormones and chemicals on lipase activity, the following were used in the concentration mentioned against each in the optimal

reaction mixture: epinephrine 1 µg; insulin 40 mU; testosterone 50 µg; sodium fluoride $2 \times 10^{-2}$; 2-propanol 1% and iodoacetate (Na-salt) $10^{-5}$M and the lipase activities were determined as described above.

(d) Estimation of protein in homogenates

The protein content of the homogenates was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin (Koch-Light, England) as a standard.

# C H A P T E R – V

## RESULTS

The total lipid content of the metacercariae of C. complanatum is quite high and accounts for 8.60% and 35.77% on wet and dry weight bases respectively. Analyses of the lipid fractions reveal that the fat of the metacercariae consists of 41.85% triglycerides, 26.16% phospholipids, 12.44% free fatty acids, 11.62% cholesterol and 7.94% unidentified lipids (Table-1).

Among the phospholipid fractions (Table-2), phosphatidyl choline and phosphatidylethanolamine accounted for the major polar lipids and are made up of 40.62% and 25% respectively, whereas, lysophosphatidylethanolamine and lysophosphatidyl choline are the minor polar lipids and comprise 15.62% and 12.50% respectively. Besides, there is about 8.64% unidentified polar lipids.

The amount of neutral lipids is higher than that of polar lipids in the metacercariae and are in the ratio of 5:2. Among the other fractions, triglycerides and phospholipids account for higher values and are in the ratio of 7:5 whereas, the free fatty acids and cholesterol are present in equal ammounts.

Lipase is seen to be quite active in the metacercariae

having a specific activity of 150.80 µg FFA liberated per mg protein per hour. Further, the enzyme is sensitive to hormones and various chemicals cause inhibition and stimulation of the enzyme activity as shown in Table-3 . Surprisingly, all the hormones, which were used, stimulated the lipase activity. The maximum stimulation was seen with epinephrine, next with insulin and the least with testosterone. In case of chemicals, sodium fluoride and iodoacetate (Na-salt) stimulated the lipase activity, whereas, propanol inhibited the lipase activity completely.

# C H A P T E R - VI

## DISCUSSION

(A) Composition

Our analysis of the lipid content (Table-1) indicates that the metacercariae possess quite high amount of lipid, which is approximately about 8.6% and 35.77% of the wet and dry tissue weights respectively. Cain & French (1975) have classified adult trematodes into three categories on the basis of the total lipid content, low (1-5% of dry tissue weight), high (about 34% of dry tissue weight) and intermediate (12-13% of dry tissue weight). The high concentration of lipids suggests that this metacercaria falls in the high lipid content category of adult trematodes mentioned above. However, this high lipid content in the metacercaria is not surprising since the metacercaria closely resembles the adult except that the reproduction is lacking. This may also be one of the reasons for high lipid content in the metacercariae because it has been reported that helminths store lipids to produce eggs and it has been observed that the decreased amount of fat has been recovered from the expiled eggs (von Brand, 1941; Gsil, 1958).

The total lipid content of the metacercariae are comparable, both on the wet and dry weight bases, with the

TABLE-1

Total lipid and lipid fractions of the metacercariae of *Clinostomum complanatum*

| Content | n | % Wet wt | % Dry wt | % of Total lipids |
|---|---|---|---|---|
| | | M±SEM | M±SEM | |
| Total lipids | 4 | 8.6±0.14 | 35.77±0.58 | ——— |
| Triglycerides | 5 | 3.6±0.18 | 14.93±0.80 | 41.85 |
| Phospholipids | 6 | 2.3±0.29 | 09.39±0.51 | 26.16 |
| Free fatty acids | 5 | 1.0±0.11 | 04.46±0.47 | 12.44 |
| Cholesterol | 4 | 1.0±0.09 | 04.21±0.39 | 11.62 |
| Unidentified lipids | 5 | 0.7±0.03 | 02.82±0.04 | 07.94 |

adults of Setylopheron cotylophorum, Fasciolopsis buski, Gigantocotyle explanatum and Echinostoma malayanum (Yusufi & Siddiqi, 1976) and with the adults of Schistosoma mansoni (Smith & Brooks, 1969; Smith, Brooks & White, 1969), E. malayanum and Isoparorchis hypselobagri (Yusufi & Siddiqi, 1976) respectively. The reason for the high lipid content may be due to the prolonged survival of the metacercariae, which store and utilize lipids primarily as energy substrate (Engelbrecht & Palm, 1964). But the total lipid of Fasciola gigantica (Weinland & von Brand, 1956); F. gigantica, Gastrothylax crumenifer and Paramphistomum explanatum (Goil, 1958) have been reported low and are not comparable with our observations neither on dry nor on the wet weight basis. This disparity may be due to the different techniques employed. Further, our results of total lipid contents are not in accordance with those of G. crumenifer and Gastrodiscoides hominis (Yusufi & Siddiqi, 1976, 1977).

However, the total lipid content of this metacercaria is comparable with other helminths (Warren & Daugherty, 1937; Roberts, 1961; Goodchild & Vilar-Alvarez, 1962; Beames & Fisher, 1964) on wet weight basis and with other helminths on dry weight basis (for reviews see von Brand, 1973 and Vysotskaya & Siderov, 1973). Fractionation of the lipids showed that triglycerides account for a high percentage of the total lipids in the metacercariae but the triglyceride content of the trematodes studied by Smith & Brooks (1969), Meyer,

Meyer & Bueding (1970) and Yusufi & Siddiqi (1976, 1977) are low and are not comparable with our observations neither on the wet nor on the dry weight basis. The high triglyceride content in the metacercariae may be due to the fact that they inhabit the body cavity of the fish Trichogaster fasciatus and the body cavity of the fish is supposed to be biologically oxygen-rich environment (Smyth, 1956) and oxygen is known to be required for the biosynthesis of unsaturated fatty acids in other animals (Goldfine & Bloch, 1963).

Cholesterol and free fatty acids are present in approximately similar amounts and are in accordance with those of Dugesia dorotocephala (Meyer et al., 1970), F. buski, I. hypselobagri, G. hominis (Yusufi & Siddiqi, 1976, 1977) and with those of S. mansoni (Cicchni, Belli, Messeri & Passi, 1976), C. cotylophorum, G. crumenifer, G. explanatum, I. hypselobagri, G. hominis (Yusufi & Siddiqi, 1976, 1977) respectively. It has been reported that trematodes do not synthesize cholesterol and long chain fatty acids (Smith, Brooks & Lockard, 1970; Meyer et al., 1970), obviously the presence of cholesterol and fatty acids may be of dietary origin and which have been absorbed from the habitat in a similar manner to that shown for Hymenolepis diminuta (Frayha & Fairbairn, 1968) and also suggested for S. mansoni (Smith et al., 1970). Hence the nature of the habitat may deeply influence its relative abundance in parasites (Barrett, Cain & Fairbairn, 1970; Meyer et al., 1970).

The study of phospholipid content and its fractions

in trematodes is very meagre. Cephalin (which also includes phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol) which account for 25% of the total phospholipids and is comparable with those of D. dorotocephala (Meyer et al., 1970), F. hepatica (Oldenborg, Vugt & Gelde, 1975). Lecithin, the major polar phospholipid, accounts for 40.62% and is in accordance with those of D. dorotocephala (Meyer et al., 1970); F. hepatica (Oldenborg et al., 1975); G. hominis (Yusufi & Siddiqi, 1977). Lysolecithin accounts for 12.50% and is comparable with those trematodes studied by Yusufi & Siddiqi, (1976, 1977).

The present investigation clearly indicates that the total lipids as well as lipid fractions of the metacercariae are similar to other trematodes inhabiting different habitats. This suggests that the habitat of the metacercariae, if playing any role in the lipid metabolism, has more or less similar influence as reported in adults.

There is no information available on the lipid content of metacercariae except few qualitative reports on neutral lipids. However, the neutral lipids of the metacercariae of C. complanatum (Table-1) are identical to the metacercariae of Leucochloridiomorpha constantiae (Fried & Shapiro, 1975); Echinostoma revolutum (Butler & Fried, 1977); Cotylurus sp (Fried & Butler, 1977) and to the adults of L. constantiae (Fried & Pucci, 1976); E. revolutum (Fried & Appel, 1977; Fried & Bedderff, 1978); D. dorotocephala (Fried & Grigo, 1977).

TABLE-2

Phospholipid fractions of the metacercariae of *Clinostomum complanatum*

| Content | n | % Wet wt | % Dry wt | % of phospholipid |
|---|---|---|---|---|
| | | M±SEM | M±SEM | |
| Lecithin | 4 | 0.93±0.03 | 3.90±0.02 | 40.62 |
| Cephalin | 4 | 0.58±0.01 | 2.40±0.20 | 25.00 |
| Lysolecithin | 4 | 0.36±0.01 | 1.53±0.05 | 12.50 |
| Lysocephalin | 4 | 0.29±0.05 | 1.20±0.10 | 15.62 |
| Unidentified lipids | 4 | 0.19±0.01 | 0.81±0.05 | 08.64 |

These similarities lead us to suggest that the qualitative nature of the neutral lipids in the metacercariae and in adult trematodes remains the same.

(B) Enzymatic studies

The lipase activity, which is about 150.81 µg FFA/ mg protein/h indicates that the breakdown of triglycerides occur in the metacercariae. Lipase has also been localized histochemically in the tegument and intestine (Alam & Nizami, unpublished) which might be hydrolysing the triglycerides, at or near the body surfaces and in the intestine respectively, ultimately liberating glycerol and free fatty acids which are being absorbed by simple diffusion for re-synthesis of triglycerides probably by means of an $\alpha$-glycerophosphate pathway in the same manner as has been suggested for S. mansoni (Meyer et al., 1970). Further, 2-propanol completely inhibits this lipase activity and such inhibition has been reported from other animals (Murray, 1929). Surprisingly, however, sodium fluoride and iodoacetate (Na-salt) stimulated the lipase activity. The reason for this is not clear but Mattson & Beck (1955) have also observed the same phenomenon in the presence of sodium chloride, ammonium chloride and ammonium sulfate and suggested that either it might be due to an effect on the enzyme itself or be due to binding of the liberated fatty acids as soaps. Insulin, epinephrine and testosterone have been found to increase the lipase activity of metacercariae, a similar effect of epinephrine (Gordon & Cherkes, 1958;

**TABLE-3**

**Effect of hormones/chemicals on lipase activity of the metacercaria of *Clinostomum complanatum***

| Hormones/ Chemicals | Concentration | n | Enzyme Activity* | % Inhibition/ Stimulation |
|---|---|---|---|---|
| | | | M±SEM | |
| Normal | —— | 4 | 150.81±10.86 | —— |
| Epinephrine | 1 µg/ml | 4 | 545.41±29.38 | -261.66 |
| Testosterone | 50 µg/ml | 4 | 420.80±64.91 | -101.93 |
| Insulin | 40 mU/ml | 4 | 304.53±15.98 | -179.70 |
| Sodium fluoride | $2x10^{-2}M$ | 4 | 446.41±12.92 | -101.51 |
| Sodium iodoacetate | $10^{-5}M$ | 4 | 317.48±08.40 | -196.01 |
| 2-Propanol | 1% | 4 | Nil | -100.00 |

*Values are expressed as µg FFA liberated/mg protein/hour

White & Engel, 1958; Engel & White, 1960; Rizack, 1961) and insulin (Gordon & Cherkes, 1958) has been reported for rat adipose tissue. The stimulatory effects caused by hormones on lipase indicate that the lipase of metacercariae is hormone sensitive like that of rat adipose tissue (Chmelař & Chmelařová, 1970).

In the light of thevpresent study and with the help of available literature, the following conclusions concerning availability, absorption and possible speculative enzymatic pathways which might be operating in the lipid metabolism of the metacercariae, may be drawn. The metacercariae might be synthesizing freely the water-soluble components of complex lipids such as glycerol, choline, or inositol and forming complex lipids from various precursers. As cholesterol and long chain fatty acids are not synthesized by trematodes so they might have been absorbed from the habitat by simple diffusion. Glycerol and FFA, the hydrolysed products of triglycerides by lipase and fatty acids absorbed in the free form may enter a common pool. From this pool, the triglycerides of distinctive composition may be re-synthesized by means of an $\alpha$-glycerophosphate pathway with the help of phosphatidic acid precursor. It has been shown that the absence of a functional $\beta$-oxidation sequence in the helminths could be related either to the relative unimportance of the TCA cycle to which $\beta$-oxidation is tightly coupled or to the low environmental $pO_2$. The metacercaria understudy lives in the body cavity of a fish which is partially oxygen-rich environment.

Previous studies of the incorporation of $^{14}C$-glucose and fixation of $^{14}CO_2$ by this metacercaria indicated that this metacercaria has a functional TCA cycle. It would be of interest to determine whether this metacercaria possesses a functional/non functional $\beta$-oxidation sequence or it is absent.

As lipids are known to be synthesized in the parasite's body and stored at various regions to meet the energy requirements of their needs. Hence the high amount of lipids present in this metacercaria may be correlated with the utilization of this stored lipids for the production of energy for this unusual long surviving progenetic metacercaria, which dwells for longer time span in the body cavity of a fresh water fish. In addition, the capacity of the body to store carbohydrates is rather limited and as the metacercaria has attained full growth so it cannot store surplus proteins from the environment to be utilized in the egg production which is lacking (may be due to some physico-chemical factors or due to some trigger stimuli which are not available in the intermediate host, the fish). So perhaps the metacercaria might have actively stored lipids to be needed to produce eggs because the average metabolic energy derived from lipid is much higher, compared to carbohydrates or proteins due to the oxidation of highly reduced hydrocarbon radical attached to carboxyl ($-\overset{O}{\overset{\|}{C}}-OH$) group of fatty acids. Thus, lipids may qualitatively and quantitatively be an important source for energy requirements

in this metacercaria of C. complanatum.

A number of hormones/chemicals affect the mobilization of fats. So the chemical factors which may be of some significance in the chemical physiology of larval trematodes, the hormones of the host could be one of them. The actual mechanism of their action is not completely understood as yet but the following possibilities may be considered. Firstly, the host hormones may increase lipolysis of triglycerides by the lipase. Secondly, it may increase the release of free fatty acids. Thirdly, it may alter membrane permeability of the parasites to glucose and carbohydrate intermediates and thus may increase triglyceride synthesis in the parasites. Several hormones like epinephrine, testosterone and insulin have been tested and found to have stimulated lipolysis and caused release of free fatty acids in the metacercaria of C. complanatum in the present study. Similarly several chemicals like sodium fluoride, iodoacetate and propanol have been tested in which sodium fluoride and iodoacetate have stimulated, whereas propanol has completely inhibited, the lipase activity.

# C H A P T E R - VII

## SUMMARY AND CONCLUSION

The present dissertation is based on the "studies on the lipid metabolism of the metacercariae of Clinostomum complanatum". The metacercariae possessed considerable amount of lipids (about 35.77% dry weight). Fractionation of the lipids showed triglycerides (about 41.85% of total lipids) and phospholipids (about 26.16% of total lipids) as the major components, whereas cholesterol (about 11.62% of total lipids) and free fatty acids (about 12.44% of total lipids) were found to be minor components. Besides, there is about 7.94% unidentified lipids of total lipids. Further, phospholipid fractions by thin layer chromatography (TLC) revealed lecithin (40.62% of total phospholipids) and cephalin (25% of total phospholipids) as the major polar lipids, whereas lysolecithin (12.50% of total phospholipids) and lysocephalin (15.62% of total phospholipids) were present in small fractions. There is about 8.64% unidentified polar lipids of total phospholipids. The amount of neutral lipids is higher than that of polar lipids and are in the ratio of 5:2 respectively. The specific activity of lipase (E.C.3.1.1.3) was found to be 150.81 µg free fatty acids liberated/mg protein/h. Different concentrations of epinephrine, testosterone, insulin, sodium fluoride, and iodoacetate stimulated the

lipase activity, while 2-propanol inhibited the lipase activity completely.

In conclusion, it is suggested that further studies on the identification of unknown lipids and study of other enzymes of lipid metabolism like $\beta$-oxidation will lead to a better understanding of the lipid metabolism of the metacercariae of *C. complanatum*.

# REFERENCES

Axmann, M.C. (1947) Morphological studies on glycogen deposition in schistosomes and other flukes. J. Morphol. 80: 321-334.

Barrett, J., Cain, G.D. & Fairbairn, D. (1970) Sterols in Ascaris lumbricoides (Nematoda), Macracanthorhynchus hirudinaceus and Moniliformis dubius (Acanthocephala) and Echinostoma revolutum. J. Parasitol. 56: 1004-1008.

Bartlett, G.R. (1959) Phosphorus assay in column chromatography. J. Biol. Chem. 234: 466-468.

Beames, C.G. & Fisher, F.M. (1964) A study on the neutral lipids and phospholipids of the Acanthocephala Macracanthorhynchus hirudinaceus and Moniliformis dubius. Comp. Biochem. Physiol. 13: 410-412.

Brand, T. von (1928) Beitrag zur Kenntnis der Zusammensetzung des Fettes von Fasciola hepatica. Z. vergl. Physiol. 8: 613-624.

Brand, T. von (1941) Aerobic fat metabolism of Ascaris lumbricoides. Proc. Soc. Exptl. Biol. Med. 46: 417-418.

Brand, T. von (1973) Biochemistry of Parasites. 2nd Edition. Academic Press, New York and London.

Butler, M.S. & Fried, B. (1977) Histochemical and thin layer chromatographic analyses of neutral lipids in Echinostoma revolutum metacercariae cultured in vitro. J. Parasitol. 63: 1041-1045.

Cain, G.D. & French, J.A. (1975) The effect of parasitism by the lung fluke, Haematoloechus medioplexus, on lung fatty acids and sterol composition in the bull frog, Rana catesbiana. Int. J. Parasitol. 5: 159-164.

Carpenter, M.F.P. (1952) The digestive enzymes of Ascaris lumbricoides var. suis; their properties and distribution in the alimentary canal. Dissertation Univ. Michigan. Univ. Microfilm, Publ. No. 3729, Ann Arbor, Mich. pp. 183.

Cheng, T.C. (1965) Histochemical observations on changes in the lipid composition of the American Oyster, Crassostrea virginica (Gmelin) parasitized by the trematode Bucephalus sp. J. Invert. Pathol. 7: 398-407.

Cheng, T.C. & Snyder, R.W. (1962) Studies on host-parasite relationships between larval trematodes and their hosts. III. Certain aspects of lipid metabolism in Helisoma trivolvis (Say) infected with the larvae of Glypthelmins pennsylvaniensis Cheng and related phenomenon. Trans. Am. Microsc. Soc. 81: 327-331.

Chmelař, M. & Chmelařová, M. (1970) Multiple forms of hormones--sensitive lipase in different rat tissues. In: Enzymes and Isozymes- Structure Properties and Funmtion (Ed. Shugar, D.). Fed. Eur. Biochem. Soc. 18: 215-226.

Cicchini, T., Belli, M., Messeri, E. & Passi, S. (1976) Determination of the cercarial lipids and relations with superficial human cutaneous lipids. Annali. Sclavo Italy. 18: 720-726.

Engel, F.L. & White, J.E. (1960) Some hormonal influences on fat metabolism from adipose tissue. Am J. Clin. Nutr. 8: 691-704.

Engelbrecht, H. & Palm, V. (1964) Der endogene Glykogen-und Fettstoffwechsel in seiner Bedentung fur die Differenzierung, Lebens-und Infektions fahigkeit der Entwicklungsstadien parasiticher Wurmer. Z. Parasitenkd. 24: 88-104.

Erasmus, D.A. (1967) Ultrastructural observations on the reserve bladder system of Cyathocotyle bushiensis Khan 1962 (Trematoda:Strigeoidea) with special reference to lipid excretion. J. Parasitol. 53: 526-536.

Erasmus, D.A. & Öhman, C. (1963) The structure and function of the adhesive organ in strigeid trematodes. Ann. N.Y. Acad. Sci. 113: 7-35.

Faust, E.S. & Tallqvist, T.W. (1907) Uber die Ursachen der Bothriocephalusanamie. Ein Beitrag zur Pathogenese der Anamie auf physiolgisch-chemischer Grundlage. Arch. Exptl. Path. u. Pharmakol. 57: 367-385.

Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol..Chem. 226: 497-509.

Forster, R. & Taggart, T. (1950) Use of isolated renal tubules for the examination of metabolic processes associated with active cellular transport. J. Cell. Comp. Physiol. 30: 251-270.

Frayha, G.J. & Fairbairn, D. (1968) Lipid metabolism in helminth parasites. VII. Absorption of cholesterol by Hymenolepis diminuta (Cestoda). J. Parasitol. 54: 1144-1146.

Fried, B. & Appel, A.J. (1977) Excretion of lipids by Echinostoma revolutum (Trematoda) adult. J. Parasitol. 63: 447.

Fried, B. & Boddorff, J.M. (1978) Neutral lipids in Echinostoma revolutum (Trematoda) adults. J. Parasitol. 64: 174-175.

Fried, B. & Butler, M.S. (1977) Histochemical and thin layer chromatographic analyses of neutral lipids in metacercarial and adult Cotylurus sp. (Trematoda: Strigeidae). J. Parasitol. 63: 851-854.

Fried, B. & Grigo, K.L. (1977) Histochemical, thin layer and histographic analyses of neutral lipids in the planarian Dugesia dorotocephala (Turbellaria). Trans. Am. Microsc. Soc. 94: 530-532.

Fried, B. & Pucci, D.L. (1976) Histochemical and thin layer chromatographic analyses of neutral lipids in Leucochloridiomorpha constantiae (Trematoda) adults. Int. J. Parasitol. 6: 479-482.

Fried, B. & Shapiro, I.L. (1975) Accumulation and excretion of neutral lipids in the metacercaria of Leucochloridiomorpha constantiae (Trematoda) maintained in vitro. J. Parasitol. 61: 906-909.

Fried, B. & Shapiro, I.L. (1979) Thin layer chromatographic analysis of phospholipids in Echinostoma revolutum (Trematoda) adults. J. Parasitol. 65: 245-247.

Ginetsinskaia, T. A. (1961) The dynamics of the storage of fat in the course of the life cycle of trematodes (In Russian). Dokl. Akad. Nauk. SSSR. 139: 1016-1019.

Ginetsinskaia, T.A. & Dorofeikii, A.A. (1962) Glycogen and fat in the various phases of the life cycle of trematodes (In Russian). Vestnik. Leningard. Univ. No. 9, Biol. Ser., fase. 2: 67-81.

Goil, M.M. (1958) Fat metabolism in trematode parasites. Z. Parasitenkd. 18: 320-329.

Goldfine, H & Bloch, K. (1963) Oxygen and biosynthetic reactions. In: Control mechanism in respiration and fermentation (Edited by Wright, B.). Ronald Press, New York.

Goodchild, C.G. & Vilar-Alvarez, C.M. (1962) Hymenolepis diminuta in surgically altered hosts. II. Physical and chemical changes in tapeworms grown in shortened intestines. J. Parasitol. 48: 379-383.

Gordon, R.S. & Chekes. A. (1958) Production of unesterified fatty acids from isolated rat adipose tissue incubated in vitro. Proc. Soc. Exptl. Biol. Med. 97: 150-151.

Handel, E. van & Zilversmit, D.B. (1957) Micromethod for the direct determination of serum triglycerides. J. Lab. Clin. Med. 50: 152-157.

Harris, K.R. & Cheng, T.C. (1973) Histochemical demonstration of fats associated with intestinal caeca of Leucochloridiomorpha constantiae, Trans. Am. Microsc. Soc. 92: 496-502.

Hoskin, G.P., Cheng, T.C. & Shapiro, I.L. (1974) Fatty acid compositions of the three lipid classes of Himasthla quissetensis rediae before and after starvation. Comp. Biochem. Physiol. 47: 821-829.

Itaya, K. & Ui, M. (1965) Colorimetric determination of free fatty acids in biological fluids. J. Lipid Res. 6: 16-20.

James, B.L. & Bowers, E.A. (1967) Histochemical observations on the occurrence of carbohydrates, lipids and enzymes in the daughter sporocyst of Cercaria bucephalopsis Lacaze-Duthiers, 1854 (Digenea: Bucephalidae). Parasitology 57: 79-86.

Kent, N.H. (1963) Seminar on immunity to Parasitic Helminths. V. Antigens. Exptl. Parasitol. 13: 45-56.

Körting, W. & Fairbairn,D. (1971) Changes in $\beta$-oxidation and related enzymes during the life cycle of Strongloides ratti (Nematode). J. Parasitol. 57: 1153-1158.

Lee, D.L. (1958) Digestion in Leidynema appendiculata (Leidy, 1850), a nematode parasitic in cockroaches. Parasitology 48: 437-447.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with folin phenol reagent. J. Biol. Chem. 193: 266-275.

Lutta, A.S. (1939) The dynamics of the glycogen and fat in parasitic worms (In Russian). Uchen. Zapiski. Leningrad. Gos. Univ. Ser. 11: 129-171.

Mandlowitz, S., Dusanic, D. & Lewert, R.M. (1960) Peptidase and lipase activity of extracts of Schistosoma mansoni cercariae. J. Parasitol. 46: 89-90.

Marinetti, G.V. (1962) Chromatographic separation, identification and analysis of phosphatides. J. Lipid Res. 3: 1-20.

Mattson, F.H . & Beck, L.W. (1955) The digestion in vitro of triglycerides by pancreatic lipase. J. Biol. Chem. 214: 115-125.

McManus, D.P., Marshall, I. & James, B. L. (1975) Lipids in digestive gland of Littorina saxatilis rudis (Maton) and in daughter sporocyst of Microphalus similis (Jag, 1900). Exptl. Parasitol. 37: 157-163.

Meyer, F., Meyer, H. & Bueding, E. (1970) Lipid metabolism in the parasitic and free-living flatworms, Schistosoma mansoni and Dugesia dorotocephala. Biochim. Biophys. Acta. 210: 257-266.

Misra, U.K. (1968) Liver lipids of rats administered excessive amounts of retinol. Can. J. Biochem. 46: 697-701.

Murray, D.R.P. (1929) XXXVII. Molecular constitution and accessibility to enzymes. The effect of various substances on the velocity of hydrolysis by pancreatic lipase. Biochem. J. 23: 292-308.

Nassi, H. (1976) Trematode sterilisant Biomphalaria glabrata en Guadeloupe. Bull. Soc. Zool. Fr. 101: 1062-1063.

Oldenborg, V., Vugt, F. van & Gelde, L.M.G. van (1975) Composition and metabolism of phospholipids of Fasciola hepatica, the common liver fluke. Biochim. Biophys. Acta. 398: 101-110.

Pascoe, D. & Richards, R.J. (1970) Original paper not seen, quoted in von Brand (1973) pp. 237.

Pennoit-De Coeman, E. & Grembergen, G. van (1942) Vergelijken onderzoek van het fermentensteem bij vrijlevende en parasitaire Plathelminthen. Verh. Vlaam. Akad., Wetensch. 4: 7-77.

Rai, P. (1968) On the clinostomatid metacercaria in some of our edible fishes and remarks on the pathological significance. Ind. J. Anim. Sci. 40: 189-198.

Risack, M.A. (1961) An epinephrine-sensitive lipolytic activity in adipose tissue. J. Biol. Chem. 236: 659-662.

Roberts, L.S. (1961) The influence of population density on patterns and physiology of growth in Hymenolepis diminuta (Cestoda: Cyclophyllidea) in the definitive host. Exptl. Parasitol. 11: 332-371.

Rogers, W.P. (1941) Digestion in parasitic nematodes. II. The digestion of fats. J. Helminthol. 19: 35-46.

Sackett, G.E. (1925) Modification of Bloor's method for the determination of cholesterol in whole blood or blood serum. J. Biol. Chem. 64: 203-205.

Schmidt, F.H., Stork, H. & Dahl, K. von (1974) Photometric assay of lipase. In: methods of enzymatic analysis (Bergmeyer, H.U., 2nd ed.) vol.II. pp. 819-823. Academic Press. New York.

Siddiqui, A.A. & Nizami, W.A. (1981) Biochemical composition and carbohydrate metabolism of the metacercariae of Clinostomum complanatum (Trematoda: Digenea). J.Helminthol. 55: ?

Singh, A.N. & Virmani, C.K. (1978) Blood picture of Colisa fasciatus (Perciformes: Anabantidae) infested by metacercariae of Clinostomum piscidium (Trematoda: Clinostomatidae). Ind. J.Parasitol. 2: 131-132.

Skipski, V.P., Peterson, R.F. & Barclay, M. (1964) Quantitative analysis of phospholipids by thin layer chromatography. Biochem. J. 90: 374-378.

Smith, T.M. & Brooks, T.J. Jr. (1969) Lipid fractions in adult Schistosoma mansoni. Parasitology. 59: 293-298.

Smith, T.M., Brooks, T.J. Jr. & Lockard, V.G. (1979) In vitro studies on cholesterol metabolism in the blood fluke Schistosoma mansoni. Lipids. 5: 850-856.

Smith, T.M., Brooks, T.J. Jr. & White, H.B. (1966) Thin-layer and gas-chromatographic analysis of lipids from cercariae of Schistosoma mansoni. Am. J. Trop. Med. Hyg. 15: 307-313.

Smith, T.M., Brooks, T.J. Jr. & White, H.B. (1969) Fatty acid composition of adult Schistosoma mansoni. Lipids. 4: 31-36.

Smorodintsev, I.A. & Bobochko, K.V. (1970) Lipoids of Taenia Hynchus saginatus. Dokl. Soc. Chem. Biol. 21: 1194-1203.

Smyth, J.D. (1959) Maturation of larval pseudophyllidean cestodes and strigeid trematodes under axenic conditions: the significance of nutritional levels in platyhelminth development. Ann. N. Y. Acad. Sci. 77: 103-125.

Subrahmanyam, D. & Venkatesan, S. (1968) On the phospholipids of Ascaris lumbricoides. Comp. Biol. Chem. 25: 755-757.

Thomas, R.E. & Galliechio, V. (1967a) Carbon dioxide fixation by the metacercariae of Clinostomum complanatum (Trematoda). Comp. Biochem. Physiol. 23: 527-531.

Thomas, R.E. & Galliechio, V. (1967b) Metabolism of $^{14}$C-glucose by the metacercariae of Clinostomum complanatum (Trematoda). J. Parasitol. 53: 981-984.

Vysotskaya, R.U. & Siderov, V.S. (1973) Lipid content of some helminths from fresh water fishes. Parasitology. 7: 51-57.

Ward, C.W. & Fairbairn, D. (1970) Enzymes of $\beta$-oxidation and their function during development of Ascaris lumbricoides eggs. Dev. Biol. 22: 366-387.

Warren, Mc W. & Daugherty, J. (1957) Host effects on the lipid fraction of Hymenolepis diminuta. J. Parasitol. 43: 521-526.

Weinland, E. & Brumd, T. von (1926) Beobachtungen an Fasciola hepatica. Z. vergl. Physiol. 4: 212-285.

White, J.E. & Engel, F.L. (1958) Lipolytic action of corticotropin on rat apipose tissue in vitro. J. Clin. Invest. 37: 1556-1563.

Yusufi, A.N.K. & Siddiqi, A.H. (1976) Comparative studies on the lipid composition of some digenetic trematodes. Int. J. Parasitol. 6: 5-8.

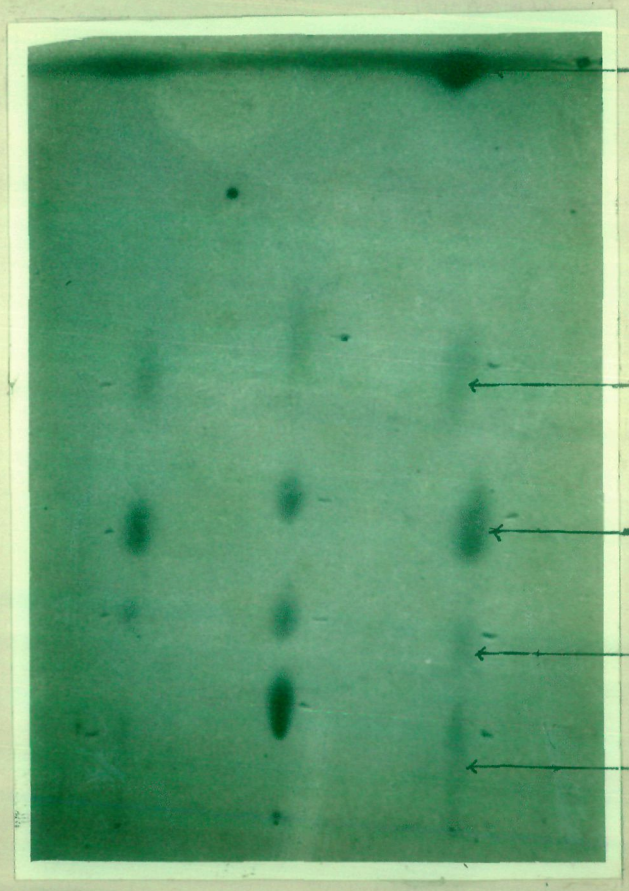
Yusufi, A.N.K. & Siddiqi, A.H. (1977) Lipid composition of Gastrodiscoides hominis from pig. Ind. J. Parasitol. 1: 59-61.

Šdárska, Z. (1964) Contribution to the knowledge of metabolic and morphological changes in the metacercariae of Echinochasmus revolutum. Věst. Čsl. Spol. Zool. 28: 285-289.

Zlatkis, A., Zak, B. & Boyle, A.J. (1953) A new method for the direct determination of serum cholesterol. J.Lab. Clin. Med. 41: 486-492.

Zöllner, N..& Kritsch, K. (1962) Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasma-lipoiden) gemeinsamen Sulphophosphovanillin-Reaktion. Z. ges. Exptl. Med. 135: 545-561.

Fig. 1. Thin layer chromatogram of phospholipid of the metacercaria of *C. complanatum*


Unknown
Cephalin
Lysocephalin
Lecithin
Lysolecithin
Host
Standard
Parasite