STUDIES ON THE ACETATE METABOLISM OF TAENIID TAPEWORMS

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E. granulosus E. multilocularis T. hydatigena

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ABSTRACT

Three taeniid tapeworms were shown to be capable of synthesizing lipids from radioactive acetate.

Echinococcus granulosus and *E. multilocularis* showed higher rates of metabolic activity than did *Taenia hydatigena*. The membranes of *T. hydatigena*, separated from their scolices, were the most active tissues of this species in the synthesis of the non-saponifiable lipids. Preparations of the entire cysticerci gave the highest yields in saponifiable lipid synthesis, although they failed in this respect to equal the combined activities of membranes and scolices when these were incubated separately.

Cholesterol was proven not to be synthesized from radioactive acetate in the three worms. Confirmative *in vivo* experiments were done on two albino mice infected secondarily with *E. granulosus*. When radioactive cholesterol was given orally to these mice, it was concentrated in the hydatid cysts proving its free passage from the host's tissues to the cysts.

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INTRODUCTION

1. General Review

The study of the enzymes of the hydatid cyst itself started with Cameron (1926). Among some other enzymes, he found lipase and oxidases in hydatid cyst tissues, but the hydatid cyst fluid was found practically inert as regards to these enzymes.

Rogers (1941) extracted lipase and esterase from *Ascaris lumbricoides* and *Strongylus edentatus*. He found these enzymes to be more active in media consisting of blood and tissue extracts of their hosts than in media from other sources. Lipase was also detected by Lee (1958) from *Leidynema appendiculata*, a nematode parasite in cockroaches. It was found to have a maximum activity at pH.7. Mandlowitz *et al*. (1960) studied the activity of lipase and peptidase extracted from the cercariae of *Schistosoma mansoni*. Tripalmitin, when incubated with these enzymes, changed from spheroidal shape to spicules and plaques which were shown to be palmitic acid.

Histochemical visualization of sites of lipase activity in tissues was described by Gomori (1946) and this technique has since been used by Bullock (1948, 1949) to detect lipase in helminths. Bullock used the Gomori technique to locate lipase in *Echinorhynchus*

coregoni, *Pomphorhyncus bulbocolli*, *Neoechinorhynchus cylindratus* and *N. emydis*.

Schwabe *et al*. (1961) demonstrated acetyl cholinesterase in hydatid tissues and suggested that this enzyme regulated the permeability of the hydatid cyst wall. A quaternary ammonium compound was detected too and could possibly be a choline-related compound such as lecithin.

Two contradictions in the study of the enzymes of *Echinococcus granulosus* larval stage were seen in the results of DeLey and Vercruysse (1955), and Agosin and Aravena (1960). The first group of workers could not find the two enzymes, glucose-6-phosphate dehydrogenase and gluconic acid-6-phosphate dehydrogenase, in the larval stage of *E. granulosus* while Agosin and Aravena found all the enzymes of pentose phosphate pathway in these tissues.

Considering the chemical phase of the literature, Flössner (1925) detected in hydatid cyst fluid glycogen plus valeric acid as the only fatty acid. Čmelik (1952) worked on the chemical composition of the germinal and laminated membranes of the hydatid cyst. He found fat in the ether extract of these membranes; the unsaponifiable lipid was cholesterol, the saponifiable fatty acids

were likely to be oleic acid due to the iodine number. The first work published on the metabolism of _E. granulosus_ was by Agosin _et al._ (1957). It was about the general chemical composition and respiratory reactions. Oxygen consumption and CO_2 production were determined, but the sources of this CO_2 were not established. The aerobic and anaerobic gaseous exchanges were highly sensitive to inhibitors of glycolysis. The ionic composition of the medium was shown to have a considerable influence on the respiratory rate with hydatid cyst fluid and Ringer's solution sustaining the respiration best. As to the chemical composition of the scolices, it was found that they contained large amounts of protein and smaller amounts of lipids. Two polysaccharides were found to be stored: glycogen and a polysaccharide containing galactose, glucosamine and possibly glucose. Agosin and Repetto (1963) continued their research on the metabolism of _E. granulosus_. Using isotopic tracers as labeled CO_2 and acetate - 2 - C^{14}, they demonstrated the tricarboxylic acid cycle and detected the synthesis of lipids and phospholipids; but no attempts were made to further identify the several compounds present in the various lipid fractions obtained.

Kilejian _et al._ (1962), using infra red spectroscopy to detect the chemical components of hydatid cyst tissues noted

absorption bands characteristic of lecithin and cholesterol in lipid extracts of the laminated membrane.

Another worm extensively experimented upon for its metabolic processes has been *Ascaris lumbricoides*. Rogers and Lazarus (1949) extracted from its tissues lecithin, sphingomyelin, serine and ethanolamine containing phospholipids. Masquelier and Bailenger (1949) found in the *Ascaris* extracts a hemolytic substance of lipid nature in the inactive lipidoproteic complex form. Ameel (1950) and Eliott (1954) described the relationship of aging, food reserves and infectivity of some ascarid larvae with fat concentration in these larvae. Fairbairn and Passey (1956), in their studies on the development of *Ascaris* eggs, found that the egg's fat was used for the synthesis of carbohydrates which were identified as glycogen and tréhalose, a non-reducing disaccharide. In a detailed study on the biochemistry of *Ascaris*, Fairbairn (1957) identified from lipids, volatile acids and triglycerides. The volatile acids consisted of acetic acid, propionic acid, C_4 and C_6 acids that were not identified in detail, C_5 acids of n-valeric acid, dl-α- methyl butyric acid and cis-α- methyl crotonic acid (tiglic). The non volatile acids found in tri-glycerides were not identified. He also found phospholipids (lecithins, cephalins and sphingomyelins), and

sterols, both saturated and unsaturated (including cholesterol). Bird (1957) studied the chemical composition of *A. lumbricoides* cuticle and found it to contain lipids in its outermost layer. Saz and Vidrine (1959) found out that succinate could be a precursor of some volatile fatty acids in *A. lumbricoides*.

Von Brand (1939) seperated quantitatively the ether extracts of *Macracantherhynchus hirudinaceous* (Acanthocephala) and found it to be of phospholipids, cholesterol, saturated and unsaturated fatty acids. Von Brand *et al*. (1951) found that the axenic larvae of *Trichinella spiralis*, converted glycogen under aerobic and anaerobic conditions, to valeric acid. Smaller amounts of C_6, C_4, C_3 and C_2 acids were also found. These authors further found that lipid did not disappear from the larvae under anaerobic conditions. However under aerobic conditions, on the contrary, starving larvae lost about 21% of the lipids found initially. It was assumed that a relatively large fraction of the consumed oxygen served to oxidise these lipids. The same group of workers (von Brand *et al*. 1952) found that the major part of the oxygen used was for the oxidation of lipids, and this process may be of importance for the motility of the larvae.

Warren and Daugherty (1955, 1957) studied the lipid metabolism of the rat tapeworm *Hymenolepis diminuta.* They reported that both the type of host and its diet were effective in determining the total fat content of the parasite, and the nature of the lipid material present. The weight of the worm had no effect on fat deposition. Thompson *et al.* (1960) worked on extracting the unsaponifiable lipids of *Taenia taeniaeformis* and *Moniezia*. After saponification and extraction with ether, they eluted the non-saponifiable portion with benzene - petroleum ether. Cholesterol and 7-ketocholesterol were found in *Moniezia* spp., while *T. taeniaeformis* had cholesterol only.

Histochemical demonstration of lipids of the hydatid cyst was first made by Cameron and Fitzpatrik (1925) and Coutelin (1931). Cholesterol was found to be abundant in the fluid, and faint in the endocyst, scolices and the cyst wall. Fragments of brood capsules showed the presence of fatty substances in the cytoplasm and the nucleii.

Chandler *et al.* (1952) studied fat deposition in *Hymenolepis diminuta* taken from castrated and intact male rats. Histochemical examinations of these worms showed a marked increase in the deposition of natural fat in the tissues of the worms removed from castrated hosts. Voge (1960) applied fat stains to paraffin and frozen gelatin sections of the cysticercoids of *H. diminuta*. Fat

was detected in the peripheral tissue layer, the wall, the scolex, and the cavity proper of the cysticercoid. Different areas of the cysticercoid showed positive stain reactions to a cerebroside and to cholesterol.

Bullock (1948, 1949) studied histochemically the fat distribution in the acanthocephalas *Echinorhynchus coregoni*, *Pomphorhynchus bulbocolli*, *Neoechinorhynchus cylindratus* and *N. emydis*. Phospholipids, cholesterol, true fat, fatty acids and lipase were localized in the muscles and the subcuticula of these species. Haley and Bullock (1952) detected histochemically small amounts of fatty materials in the cement glands of these same acanthocephala.

Hannantha (1959) studied the fat distribution in the Mehlis' gland and the egg shell of *Fasciola hepatica*. He found, by staining methods, both phospholipids and neutral fat. One year later the same author found that the Mehlis' gland of *Fenestrocephalus ganapatii* is positive to all the phospholipid stains and is also strongly positive to the periodic acid - Schiff technique. The pigment in the digestive tube of *Ascaris lumbricoides* was found to contain a lipoid complex and fatty acids as detected histochemically by Carbonell and Apitz (1959).

Lee (1960) stained tissue sections of *Thelastoma bulhõesi*, a nematode of cockroaches, to study the distribution of fat. The chief storage areas of fat were the dorsal, ventral and lateral lines, the intestine, oocytes, oogonia and ova of the worm. Giovannola (1936) found fat, by means of Sudan III to be in those stages of nematodes that precede a period of less nutrition or of fasting or in complete sexually developed stages.

2. Scope of the Investigation

The taeniid tapeworms used in this study were *Echinococcus granulosus*, *E. multilocularis*, and *Taenia hydatigena*. It may be recalled that the hydatid cyst is the larval stage of the tapeworm, *Echinococcus granulosus*. It is located in the liver, lungs and in other organs of the body of its intermediate hosts (cattle, sheep, man, etc.). The definitive host is the dog or wolf. The cyst, which grows continually, may be either sterile or fertile depending on the absence or presence of scolices. Scolices form by budding from brood capsules which arise from the germinal membrane, the inner most membrane of the cyst wall. The hydatid cyst wall has, besides this layer, a hyalin laminated layer consisting largely of a mucopolysaccaride (Kilejian *et al.* 1961, 1962), and an outer fibrous layer elaborated by the host.

Unlike *E. granulosus* which produces large single cysts, *Echinococcus multilocularis* forms an aggregate of innumerable

small cysts. These proliferate by exogenous budding. This alveolar hydatid larva appears as small, irregular cavities with thin and irregular hyaline membranes, covering a germinal membrane. The larval stage of _Taenia hydatigena_ is a cysticercus found usually on the omentum of sheep. It has a scolex, a neck and a bladder surrounded by a translucent membrane presumably of host origin.

The hydatid cyst, the alveolar cyst and the cysticercus of these three tapeworms all contain fluids that appear in chemical composition to be similar to serum or cerebrospinal fluid. They represent a mixture of metabolic products of the parasite in a transudate of the host's body fluids.

In view of the presence of lipids in these worms, the question arises whether these substances are synthesized by the parasites or absorbed from the host. Since acetate is the starting precursor for lipids, it was attempted to investigate the role of this compound in the metabolism of _E. granulosus_, _E. multilocularis_ and _T. hydatigena_.

In addition the oxidative capacity of these worms was evaluated.

MATERIALS AND METHODS

1. Parasite Material

For *Echinococcus granulosus*, viable scolices and brood capsules varying in weight from two to four grams were collected from bovine hydatid cysts obtained from local abattoirs within a few hours of slaughter of their hosts. They were washed several times with physiological saline solution by suspension and sedimentation, then used for the metabolic processes. Batches of lyophilized scolices weighing 0.3043 gm., 0.3031 gm. and 1.361 gm. respectively were used also.

For *Echinococcus multilocularis*, two to six grams of scolices and brood capsules were obtained from *Microtus arvalis*, *Meriones tristrami*, *Gerbillus gerbillus* and albino mice which were infected secondarily in the laboratory. The original strain was received from Dr. Hans Vogel, Tropeninstitut, Hamburg, Germany.

For *Taenia hydatigena*, the cysticerci were collected from the omentum of sheep slaughtered in local abattoirs within a few hours of their use. These cysticerci were freed from the hostal membranes. Their bladders were emptied and the parasites were washed several times with physiological saline solution. For the metabolic processes, sometimes 50 gm. of whole cysticerci were used, and sometimes only 2 to 6 gm. of the scolices only. In other experiments,

the membranes and necks were used sliced or unsliced. Different batches varied in weight from 6 to 20 gm. Homogenates of scolices, necks and membranes were also utilized. Homogenates were prepared in a Teflon-glass motor driven tissue grinder at ice bath temperature. The medium was Kreb-Ringer's phosphate buffer (Krebs and Henseleit 1932).

2. *In vivo* Experiments

In vivo experiments were run on two albino mice infected for twenty seven months with *E. granulosus* scolices obtained from bovine hydatid cysts. These mice were fed for a week on pellets soaked with radioactively labeled cholesterol (26 - C^{14} of 0.3 μ mole equivalent to five million counts per minute). Subsequently the mice were maintained on normal pellets for two days and then sacrificed. The cysts were carefully separated and washed with saline. The washings were added to the tissue free from cysts. The cysts from one mouse weighed 50 gm. and the cyst-free mouse carcass 27 gm. In the second mouse the cysts weighed 22.5 gm. and the cyst-free body 31.5 gm.

Each portion, the cysts and the mouse tissues, were ground separately for 15 minutes in a Waring Blender under cool conditions. The suspending solution was 10% KOH in 70% methanol. They were then boiled separately for fifteen minutes. Then each was extracted with 200 ml. ether in 25 ml. portion. The ether extract in each was

washed as described above and the total cholesterol determined. The digitonide was formed, weighed, suspended and counted and the specific activity of each was calculated.

3. Incubation

The apparatuses used for incubating the tissues were different in design in different groups of experiments but of some principle. The first apparatus consisted of a series of tightly closed test tubes connected to each other by glass tubing and rubber connections. It was supplied by a pump to force in the air (see Figure 1).

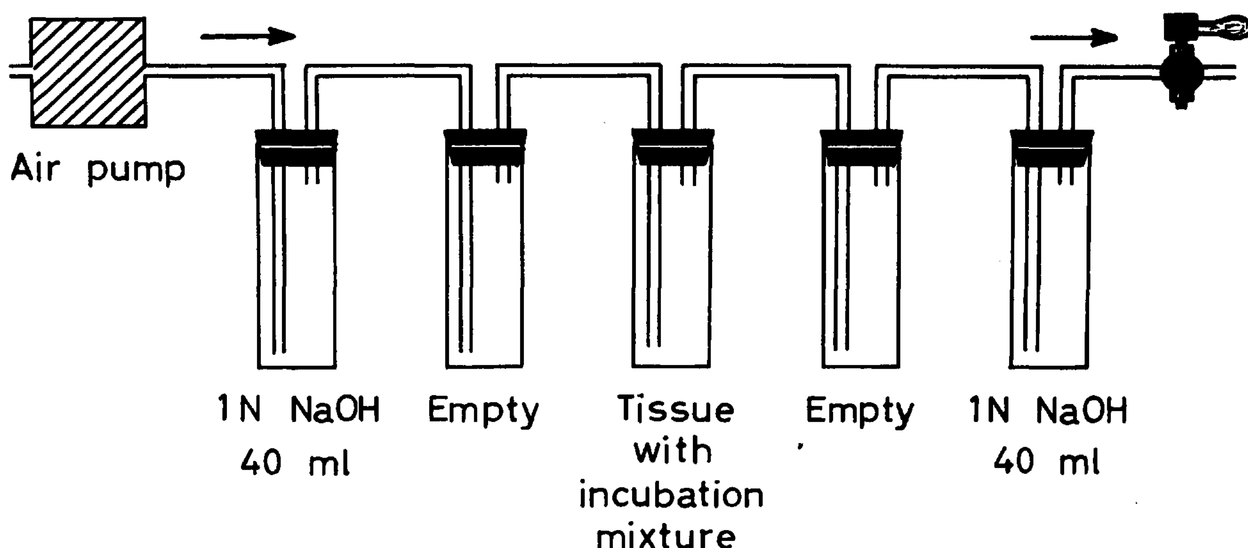


Figure 1. Initial Reaction Apparatus.

The incubation in this apparatus was at room temperature.

- The second apparatus used was a single container tightly closed with a screw cap pierced at the border. A circular piece of rubber was well seated under the cap to make it air tight. The hole in the cap was used to introduce the sulfuric acid at the end of the incubation period.

Inside the container was hung a small beaker on a tripode of polyethylene fit inside the container as shown in Figure 2. This beaker contained the alkaline. The main compartment was for the incubation mixture. Incubation in this apparatus was at 37° in an automatic water bath shaker.

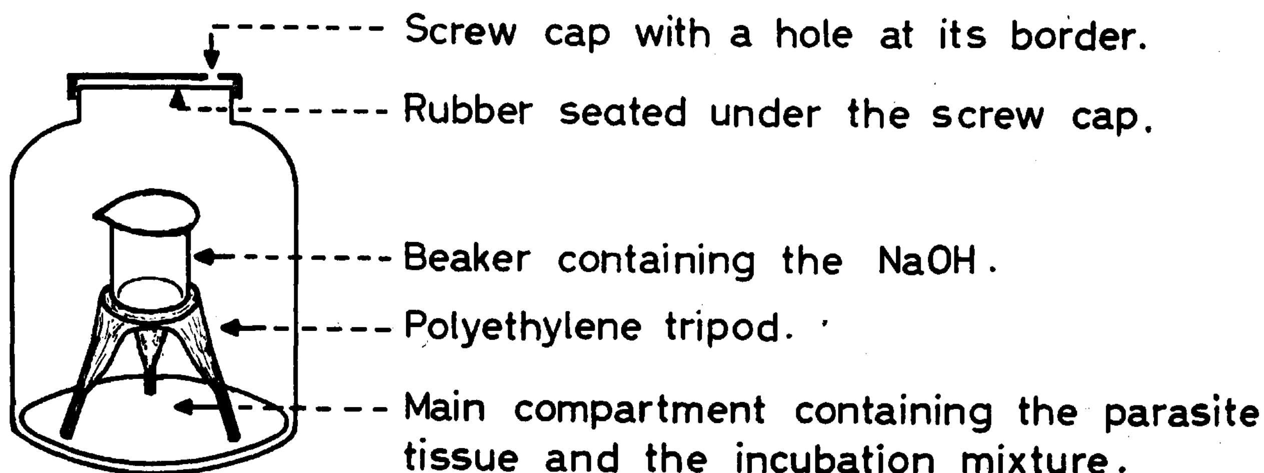


Figure 2. Second Reaction Apparatus.

The third apparatus consisted of a single side arm Warburg flask of 125 ml. capacity. The side arm of the flask was closed by a rubber diaphragm cap for introduction of the sulfuric acid at the end of the incubation period. The center well of the flask was filled with 1 ml. NaOH to trap the respired CO_2. The whole flask was connected to the Warburg manometer, the flask end of which was plugged. The parasite tissues and the incubation mixture were put in the main compartment. The flask was shaken in the Warburg bath at a constant temperature of 37°C. The period of incubation was 4 to 5 hours after which the sulfuric acid was introduced into the side arm and shaking was continued for five more minutes.

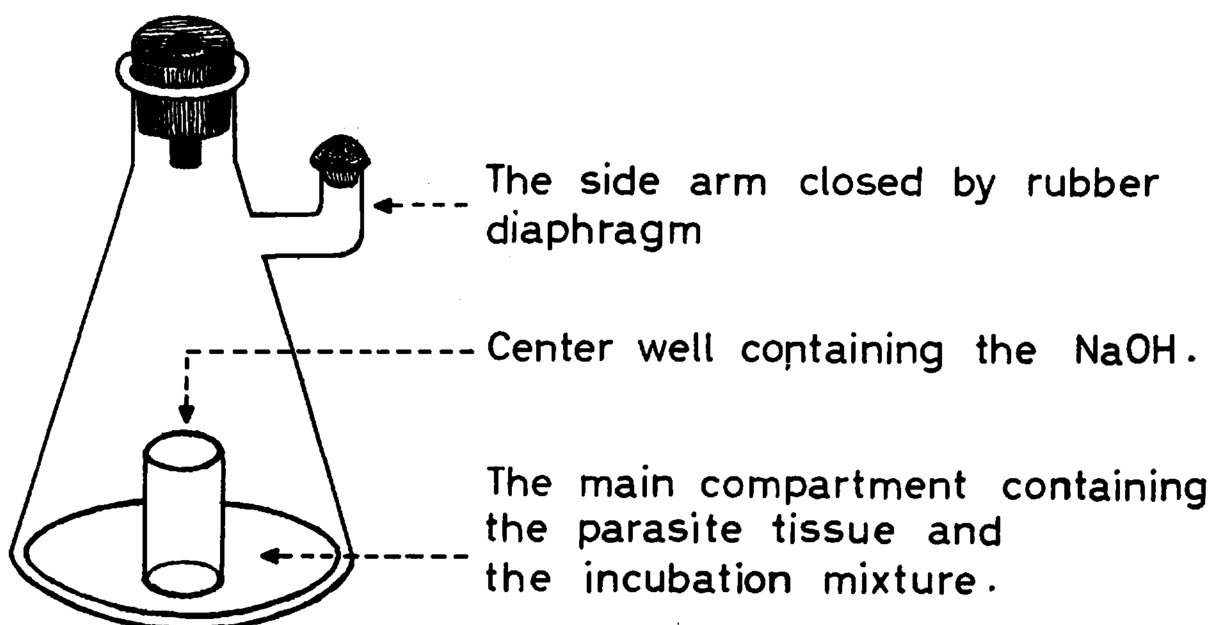


Figure 3. The Warburg Apparatus.

The incubation mixture consisted of sodium acetate-1-C^{14} in a concentration varying between 0.5μ mole to 1μ mole (equivalent to 1.2 million to 2.4 million counts per minute) depending on the amount of tissue used. Added to it were 0.1 ml. of 0.01 M sodium acetate and 0.5 ml. of antibiotic solution consisting of 500 units of penicillin and 0.5 mg. of streptomycin. The buffers used were disodium monohydrogen phosphate and potassium dihydrogen phosphate solution of pH 7.4 (Clark, 1920) and Kreb-Ringer's phosphate solution of pH 7.4 (Kreb and Henseleit, 1932). A third buffer consisted of 100 ml. of 2% stock solution (2.88% NaH_2PO_4 and 12.5% Na_2HPO_4) to which was added 0.85 gm. NaCl. The pH of this buffer was 7. The volume of the buffer used each time varied from two to five ml. depending on the amount of tissue used.

To trap the respired labeled CO_2, 2 ml. of 3N H_2SO_4 were introduced into the incubation mixture and 1-2 ml. of 2N NaOH were used in the compartments to trap the liberated CO_2.

4. Isolation of Saponifiable and Non-Saponifiable Lipids: The tissue alone after being separated from the incubation mixture or both the tissue and the incubation mixture were saponified with 10% NaOH in 70% methanol under reflux for 4 to 5 hours. Then the non-saponifiable lipid was extracted with 100 to 150 ml. of diethyl ether in portions of 25 ml. This ether extract was then washed several times with distilled water, followed by 0.1 M $KHCO_3$. Then it was dehy-

drated with dried powder of $MgSO_4$. One ml. of the washed ether extract was plated on a stainless steel planchet, dried under infra red light and counted for its radioactivity in an isotopemeter (Nuclear - Chicago Model 151 A).

The total cholesterol content was determined colorimetrically by the method of Zlatkis *et al*. (1953).

Then the ether extract was concentrated to about 10 ml. and 0.5% digitonin solution (500 mg. digitonin in 100 ml. of 50% ethyl alcohol) added in sufficient amount to precipitate all the cholesterol as digitonide. The supernatant ether portion was saved for column chromatography. The digitonide was centrifuged, washed with distilled water, absolute ethyl alcohol, and acetone-ether (1:1). After it was dried, it was weighed and 0.5 mg. was suspended in absolute alcohol, plated on a stainless steel planchet and counted for its radioactivity.

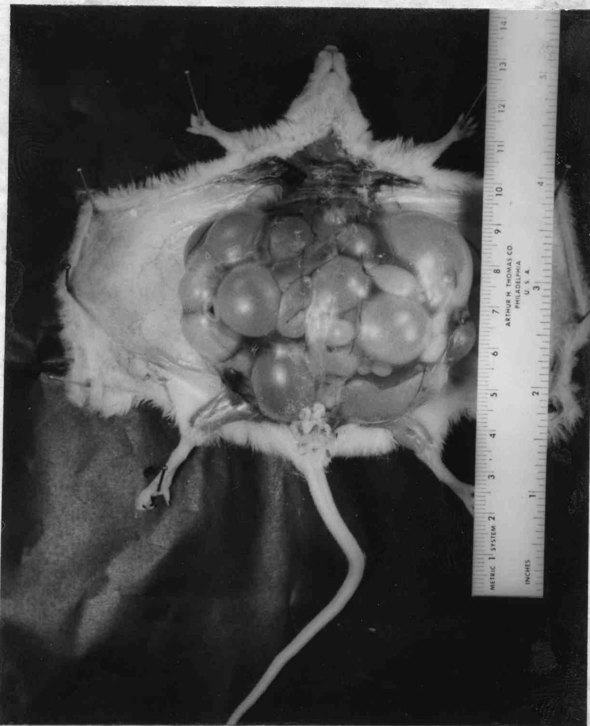
The ether cholesterol-free portion was eluted through an aluminum hydroxide dried powder washed successively with 2% acetic acid, acetone and petroleum ether and packed in a burette to a height of 15 cm., with pieces of glass wool at the outlet and on the top. Once the ether sank completely into the column, elution started with 200 ml. petroleum ether collected in 50 ml. portions, then with 200 ml. of acetone ether (1:1) collected also in 50 ml.

portions. One ml. of each portion was plated and counted for radioactivity.

To isolate the saponifiable lipids, the incubation mixture (after it was extracted with ether) was made acidic with 3N H_2SO_4 to pH 2-3. This was then extracted with 100 to 150 ml. of normal heptane in portions of 25 ml. Heptane is a long chain fatty acid solvent. The heptane extracted was washed with water, then 1 ml. was plated and counted for its radioactivity.

5. <u>Controls</u>

A control was run for each of the above experiments. Parasitic tissue was boiled for 15 minutes and handled as in the test sample.



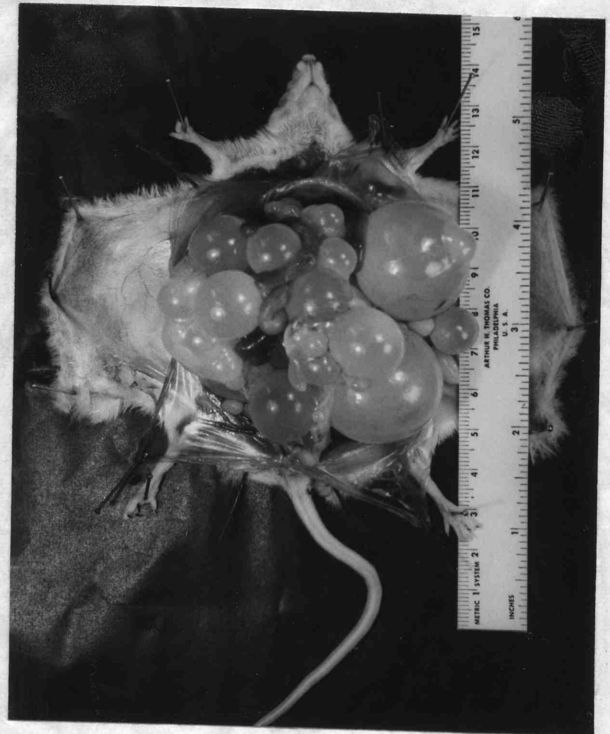
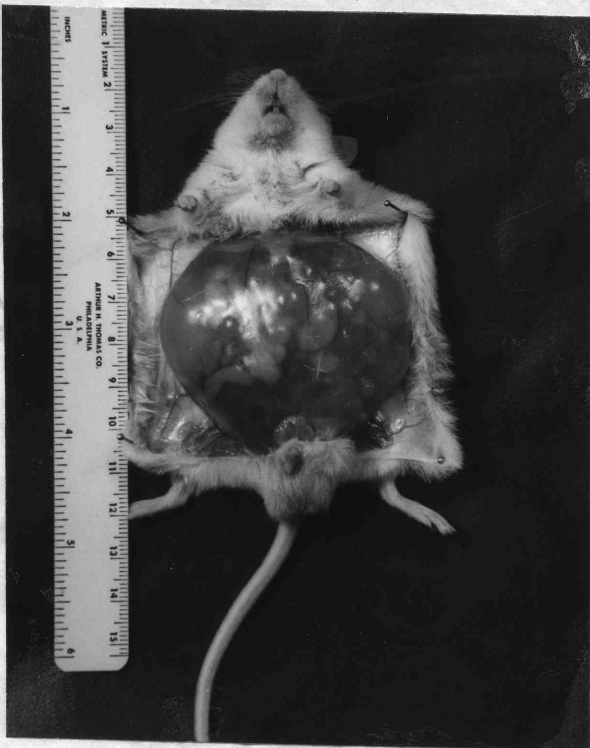


Figure 4. First mouse given radioactive cholesterol orally. The hydatid cysts are exposed.



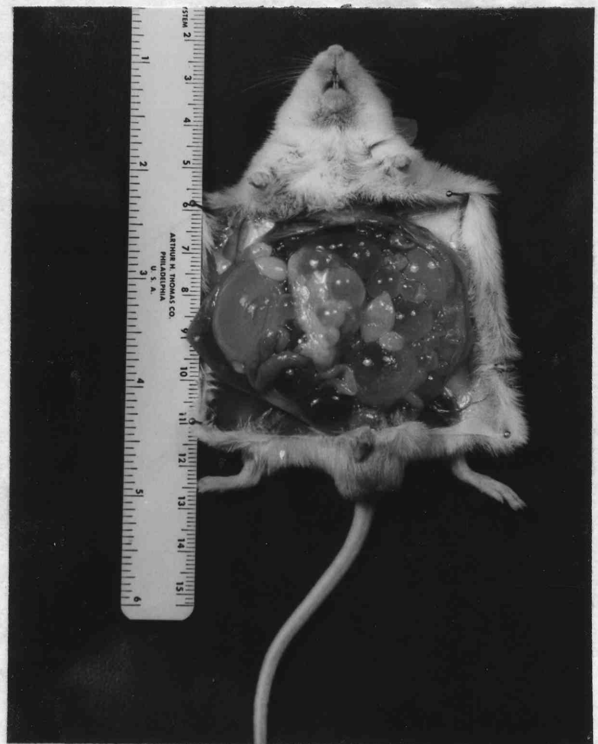


Figure 5. Second mouse given radioactive cholesterol orally. The hydatid cysts are exposed.

RESULTS AND DISCUSSION

1. Metabolism of Acetate in the Taeniid Tapeworms:- Several studies on E. granulosus have shown the presence of glycolysis and energy yielding pathways (Agosin and Repetto, 1963). However comparative studies of the biosynthesis of lipids in this cestode and others have not been reported. Consequently we sought to outline the general metabolic pattern of E. granulosus, E. multilocularis and T. hydatigena.

Data presented in Table 1 show that the fresh scolices of E. granulosus and E. multilocularis catalyzed the oxidation of acetate into CO_2. Furthermore acetate was incorporated into both the saponifiable and non-saponifiable lipids of these cestodes. Similar results, to be reported later, were obtained with T. hydatigena.

Table 1

Acetate Metabolism of E. *granulosus* and E. *multilocularis*

(Results are expressed in counts per minute per gram of tissue incubated)

Type of Tissue	CO_2	Non-saponifiable Lipids	Saponifiable Lipids
E. *granulosus*	33,000	2000	9000
Fresh Scolices	34,000	2120	200
	80,000	7000	3000
	82,000	6000	1500
	200,000	2000	400
E. *multilocularis*			
Fresh Scolices and brood capsules	53,000	1000	300
	101,000	2225	13000

Each line represents a separate experiment where the scolices and brood capsules were incubated for 4 to 5 hours with radioactive acetate, CO_2 and lipids isolated. See the text for details.

2. Stability of Scolices:- In view of the limited and irregular supply of fresh scolices of *E. granulosus* it became of interest to determine whether fresh scolices could be lyophilized and stored for subsequent use. Fresh scolices when available were immediately lyophilized in the Virtis machine (Model 145 LP-MR and 145 MR-30) and stored at -20°C. Within a period of one month, one gram of lyophilized scolices was collected. It may be noted here that one gram of dried lyophilized scolices is equivalent to 14.5 grams of wet fresh scolices. When these lyophilized scolices were incubated with C^{14}-acetate under the standard conditions of assay, the radioactivity in CO_2, non-saponifiable lipids and saponifiable lipids was: 560,000, 2,200 and 1,188 counts per minute per gram of dry tissue respectively. In a duplicate experiment the values were 542,000, 4,125 and 330 respectively.

3. Studies on *Taenia hydatigena*:- Incubation of cysticerci with C^{14}-acetate indicated that this cestode was also capable of metabolizing acetate into CO_2 and lipids (Table 2). Since it was possible to separate the scolex of this organism from its membrane, it was of interest to determine the metabolic activity of each tissue separately. Table 2 shows that the membranes were incapable of synthesizing saponifiable lipids. Apparently this

capacity was limited only to the scolices. However, it is interesting to note that although the relative efficiency of each fraction varied, nevertheless the combined activities of the scolices and the membranes had a synergistic effect.

Table 2

Acetate Metabolism of *T. hydatigena*

(Results are expressed in counts per minute per gram of tissue incubated).

Experiment	Tissue	CO_2	Non-Saponifiable Lipid	Saponifiable Lipid
1	Cysticerci	1000	80	250
	Scolices	3000	153	150
	Whole Membranes	2750	1200	0
2	Cysticerci	3709	250	465
	Scolices	4500	360	250
	Whole Membranes	10833	356	0

The weight of the cysticerci incubated is equal to the sum of weights of scolices plus membranes. Conditions for incubation are the same as Table 1.

4. **Homogenates of *T. hydatigena*:-** Homogenates of scolices and membranes were separately prepared as described in "Materials and Methods". Whereas the incorporation of acetate into lipids by the homogenate of membranes was of the same order of magnitude as that for the sliced membranes, the rate of CO_2 production was much lower than that of the sliced membranes. On the other hand the homogenates of the scolices were practically inactive. These results are shown in Table 3.

Table 3

Acetate Metabolism of Homogenates and Sliced Membranes of *T. Hydatigena*

(Results are expressed in counts per minute per gram of tissue incubated)

Type of Tissue	CO_2	Non-Saponifiable Lipids	Saponifiable Lipids
Sliced Membranes	28,690	200	117
Homogenates of Scolices	300	Negligible	Negligible
Homogenates of Membranes	1,500	200	180

The conditions for incubation are the same as Table 1.

5. <u>The Nature of the Non-Saponifiable Lipids</u>:- The presence of cholesterol in *E. granulosus* has been known for a long time. In view of the fact that acetate was incorporated into non-saponifiable lipids, it became of interest to determine whether some of this radioactivity was associated with cholesterol. In our studies, colorimetric determination of cholesterol indicated that the concentration of cholesterol was in the order of 3 mg. per gram of incubated tissue. Precipitation of cholesterol by digitonin from the non-saponifiable fraction, and analysis of the precipitate, indicated that cholesterol was not labeled. This was further confirmed by *in vivo* experiments, the results of which are shown in Table 4. The fact that the cholesterol from the cysts was labeled only when radioactive cholesterol was ingested by the mice, indicated that the cysts derived their sterol from the cholesterol food of the host. The difference in the specific activity of the isolated cholesterol from hosts and parasites probably reflected difference in the sizes of the respective pools or slow equilibrium across the cyst wall. In addition the specific activity of the cholesterol isolated from the tissues of the host was not necessarily a measure of the specific activity of the cholesterol pool which was in equilibrium with that of the cysts.

Table 4

Radioactive Cholesterol Passing Freely From Host Tissue to Hydatid Cysts in Albino Mice

In vivo Experiments		Weight in Grams	Total Cholesterol in mgm	Specific Activity in Counts per Minute per Gram of Digitonide
1st Mouse	Cysts	50	4.2	185,366
	Tissues	27	87.1	940,700
2nd Mouse	Cysts	22.5	7.2	972,225
	Tissues	31.5	86.4	2,040,000

See the text for details.

6. Column Chromatography of the Non-Saponifiable Lipids: Chromatography of non-saponifiable lipids on alumina indicated that at least two major components were present. This is shown in Table 5. Identification of these fractions was precluded by the extremely small amounts present.

Table 5

The Nature of the Non-Saponifiable Lipids

Counts per minute per gram of tissue incubated.

Biological Source	Total Non-Saponifiable Lipids	Petroleum Ether Fraction*	Acetone Ether Fraction*
E. granulosis	2000	600	400
Fresh Scolices	2120	1050	950
E. granulosis			
Lyophilized Scolices	2200 4125	1400 2850	700 1200
T. hydatigena			
Cysticerci	80 250	55 165	35 55
Scolices	158 360	134 186	68 100
Whole Membranes	1200 356	700 200	400 125
Sliced Membranes	150 250	80 153	63 50

* Each fraction was collected in 200 ml. solvent in 4 portions of 50 ml. each. See the text for details.

CONCLUSION

Although quantitative comparison of the metabolic activities of the three worms was not possible because of biological variations and the limited information available, nevertheless certain trends were discernable. For example, the general metabolic activity of E. granulosus and E. multilocularis as measured by CO_2 production and lipid synthesis was higher than that of T. hydatigena. The extent of acetate incorporated into lipids was much lower than that of acetate oxydized into CO_2.

The CO_2 produced by 1 gm. of E. granulosus fresh scolices incubated for four hours at standard conditions, accounted for 7% of the added acetate. On the other hand 0.4% of the acetate was incorporated into total lipids of this organism. In E. multilocularis the CO_2 produced per gram of fresh scolices and brood capsules incubated for four hours at standard conditions, accounted for 6% of the added acetate. And the acetate incorporated into total lipids of this organism was 0.7%.

In T. hydatigena the CO_2 produced per gram of cysticerci incubated for four hours at standard conditions, was 0.2%. The total lipids synthesized from the acetate was 0.04%. The combined activities

of scolices and membranes of T. hydatigena (when these were incubated separately) showed synergistic results. The CO_2 produced per gram of combined scolices and membranes incubated for four hours at standard conditions, accounted for 0.8% while the total lipids synthesized from the acetate was 0.09%.

Although, acetate was incorporated into non-saponifiable lipids, cholesterol was apparently not biosynthesized from acetate by these cestodes. In vivo experiments indicated that cyst cholesterol was derived from the host.

Column chromatography indicated the potentiality of this technique for separation of the various components of the non-saponifiable lipids. Identification of these components will require large scale experiments for further investigation. Studies on the metabolic activity of the various parts of T. hydatigena raised some interesting problems, e.g. the failure of intact membranes to show significant synthesis of saponifiable lipids while their ability to oxidise acetate or incorporate it to non-saponifiable lipids was evident. Furthermore, the synergistic effect of scolices and membranes requires further investigation.

These studies also showed that homogenates of T. hydatigena and probably of others can be prepared and used for the detailed studies

of the above previously mentioned metabolic pathways.

The finding that the lyophilized scolices of E. granulosus retained reasonable activity provides a chance for having a convenient source for the use of this parasite in future metabolic studies.

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