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Lipids in the host-parasite system: Digestive gland of *Lymnaea truncatula* infected with the developmental stages of *Fasciola hepatica*

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Abstract

Effects of the presence of sporocysts, rediae and cercariae of *Fasciola hepatica* on the lipid content in the digestive gland of *Lymnaea truncatula* as well as on lipid levels in tissues of the parasites themselves were studied. Lipids were examined by means of histochemical and cytophotometric techniques. The snail's digestive gland lipid level was found to be almost halved in 20 days post infection; a more then 80% reduction being visible after the subsequent 40 and 60 days. The loss of lipids in the digestive gland of the infected snails point at mobilisation of lipid energy reserves to compensate for the deficiency of carbohydrates, used by these parasites. The parasite tissues such as tegument, pharynx, suckers and germ balls showed considerable lipid contents and were metabolically active. It supports the hypothesis that lipids are used as energy source by developmental stages of this parasite.

Key words

Lipids, *Lymnaea truncatula*, digestive gland, sporocyst, redia, cercaria, *Fasciola hepatica*

Introduction

Among various lipid classes, neutral lipids (acylglycerols) are the major component of reserve fat in animal cells. Neutral lipids are highly energy-rich and can be stored in an almost water-free form as intracellular droplets suspended in the cytoplasm. As a source of energy, neutral lipids are extremely important in pulmonate snails (Duncan *et al*. 1987, Stuart and Ballantyne 1996, Klobucar *et al*. 1997). Triacylglycerols, the component lipids, are initially hydrolysed to free fatty acids which are, in turn, oxidised in the fatty acid cycle within the mitochondrial matrix (Voogt 1972, Tripathi and Singh 2002).

The sequence and details of intracellular lipid hydrolysis in pulmonates are relatively poorly known. Normally, however, no extensive accumulation of fatty acids is observed; fatty acids – like other products of lipid hydrolysis – are toxic (Cheng and Snyder 1962, Klobucar *et al*. 1997). Most likely, the intracellular lipid hydrolysis rate is tuned to the rate of fatty acid utilisation (Cheng 1965, Southgate 1970, Kumari *et al*. 1999).

There is much less information on accumulation and utilisation of lipids in trematodes, particularly in their larval forms; the information available is frequently contradictory. For example, Ginetsinskaya and Dobrovolskiy (1966) and Reznik (1968) have expressed a view that trematodes accumulate lipids as a "ballast" component which is either excreted or packed as a product undergoing no further decomposition. Other authors (Erasmus 1972, Barrett 1981, Barrett and Saghir 1999, Świderski and Mackiewicz 2004) are of the opinion that at least some parasites, particularly their free living stages and those living in intermediate hosts, can utilise lipids as an energy source.

The lipid-related issues of the relationship between a snail *Lymnaea truncatula* and larval stages of a parasite and its hosts have not been studied so far. This work was thus aimed at determining if, and to what degree, the presence of sporocysts, rediae, and cercariae of *Fasciola hepatica* in a snail affects the

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lipid content in the digestive gland (hepatopancreas) of the latter, and if the parasites concurrently accumulate lipids.

Materials and methods

The study involved the snail, *Lymnaea truncatula*, cultured in our laboratory. The snails were infected with *F. hepatica* miracidia obtained *in vitro*. An average snail received 10 miracidia. The study involved a total of 80 snails, 20 of which constituted a control (parasite-free individuals). The remaining infected snails were analysed after 20, 40, and 60 days post infection (dpi). Control snails were kept under conditions identical to those to which the infected gastropods were exposed, and both groups were analysed concurrently. Fresh tissues were frozen in dry ice and sectioned at 8 μm thickness in a cryostat.

Lipid reactions were performed with Oil Red O and with Sudan Black B (Pearse 1968). The cryostat cut slices were fixed in Baker's fluid and stained with Oil Red for 15 min and with Sudan Black for 30 min. Histochemical assays were performed on the snail digestive gland (hepatopancreas) and on the parasites; the location and intensity of staining reactions were observed and assessed under the microscope. The histochemical changes were quantitatively analysed in a cytophotometer (Chayen *et al*. 1969). Cytophotometric assays were run by taking 10 measurements of the digestive gland secretory cells in both, control and experimental groups as well as in the parasites, i.e. sporocysts, rediae and cercariae. From the results, the mean value of extinction was calculated and multiplied by the area of a particular cell, which yielded values corresponding with the relative quantity of the lipid studied. The data obtained were conventionally expressed in work units (WU) and treated statistically using parametric Student's t-test or nonparametric U Mann-Whitney test. Lipid measurements were taken at 580 nm wavelength.

Results

The highest amounts of lipids were found in the digestive gland secretory cells of the control (parasite-free) snails, with lower amounts being recorded in the haemocoel and in the calcareous cells. Fine lipid droplets were uniformly distributed in the secretory cell cytoplasm. The droplets were somewhat larger and localised at one pole of the calcareous cells. In the haemocoel the lipid droplets were also rather large. Cytophotometric measurements showed the secretory cell lipid content to be of the order of 94–100 WU, with an average value of 98.7 WU (Table I).

Table I. Contents of lipids in *Lymnaea truncatula* digestive gland at different periods post infection with larval stages of *Fasciola hepatica*. Means 10 photometric readings in work units (WU), with standard deviation (SD) and variance (V)

Secretory cells	Mean	SD	V(%)
Control	98.7	2.3	2.3
20 dpi	43.4	10.9	32.6
40 dpi	14.7	4.4	29.9
60 dpi	14.0	4.3	30.7

Table II. Content of lipids in organs of the sporocysts, rediae and cercariae at different periods post infection. Means of 10 photometric readings in work units (WU), with standard deviation (SD) and variance (V)

Organs	Infection days	Mean	SD	V (%)
		sporocysts		
Tegument	20	20.9	8.1	21.1
	40	20.8	7.2	29.2
Germ balls	20	58.6	6.1	10.4
	40	60.6	6.6	10.9
		rediae		
Tegument	20	21.1	7.8	29.2
	40	20.8	7.2	29.2
	60	22.3	7.7	28.1
Germ balls	20	59.1	8.8	14.9
	40	62.4	9.4	15.1
	60	56.7	7.5	13.2
Pharynx	20	96.8	3.7	3.8
	40	97.8	2.7	2.8
	60	96.3	4.9	5.1
		cercariae		
Tegument	40	92.2	5.1	5.5
	60	93.8	4.3	4.6
Suckers	40	97.8	1.3	1.3
	60	99.2	0.8	0.8
Cystogenous gland	40	21.4	3.6	16.8
	60	20.9	4.6	22.0

Lipid deposits in those snails assayed after 20 dpi were evidently smaller than those in the control and occurred predominantly in the secretory cells. The lipid deposits became clearly smaller in size as the infection proceeded. For example, the digestive gland of those snails examined after 40 dpi showed extensive tubule damage as a result of the activities of the rediae and cercariae present; the number of lipid droplets was much smaller than that in the control. Similar numbers and distribution patterns were observed for lipid droplets in the digestive gland of the snails examined after 60 dpi.

Cytophotometric measurements of the secretory cells showed the amount of lipids in the hepatopancreas of snails examined 20 dpi had dropped almost by half compared to the control, with more than 80% reduction being recorded in snails 40 and 60 dpi, i.e. 14.7 and 14, respectively (Fig. 1, Table I). These differences were found statistically significant ($p<0.001$).

Sporocysts showed large lipid drops in the germ balls and small droplets in the tegumental cells. In the rediae, the largest lipid deposits were evident in the pharynx and intestine, with smaller deposits visible in the germ balls. The cercariae accu-

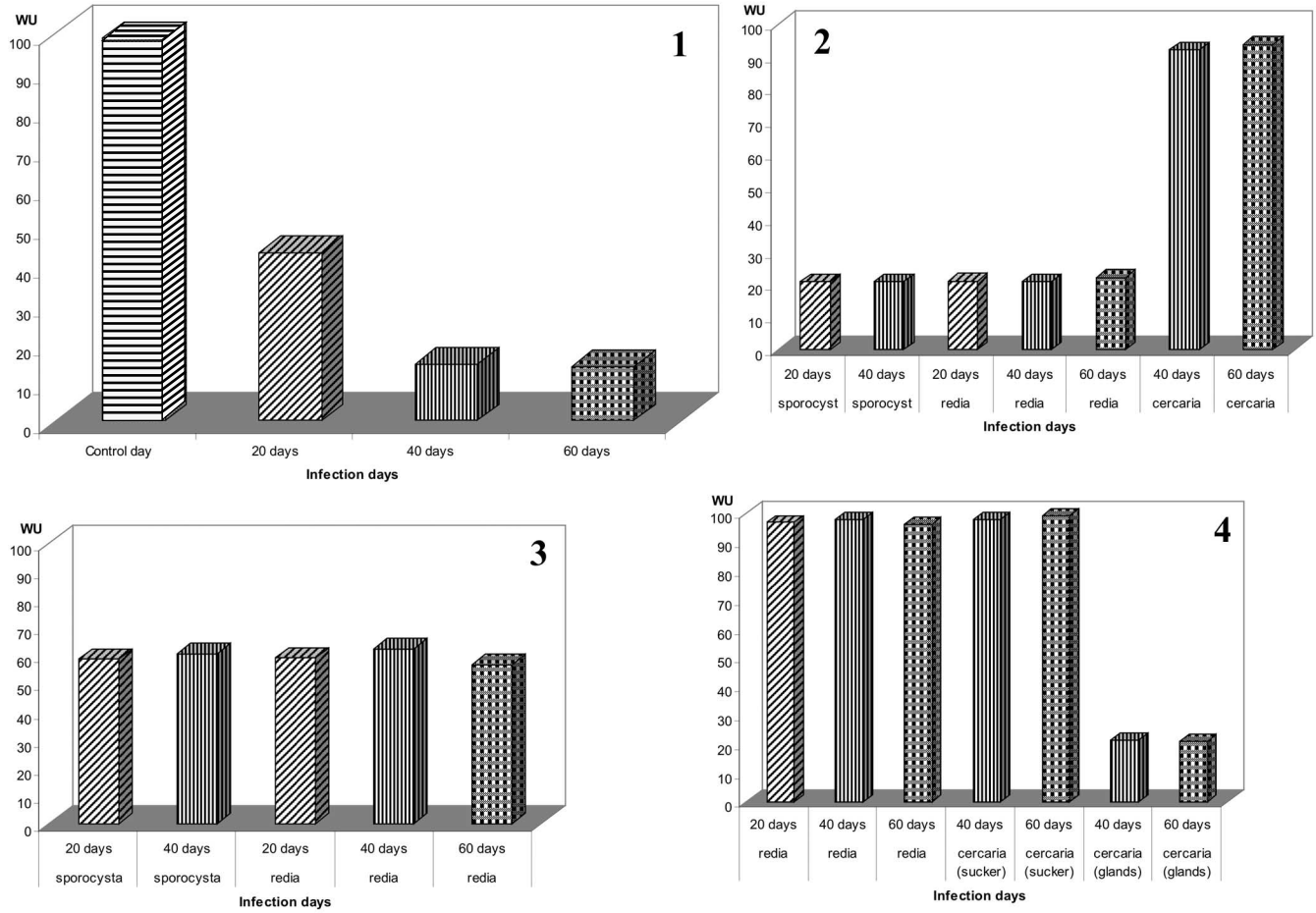

Fig. 1. Quantitative changes of lipids in digestive gland of *Lymnaea truncatula*, 20, 40 and 60 dpi in comparison with uninfected snail gland. **Fig. 2.** Content of lipids in the tegument of sporocyst, redia and cercaria, 20, 40 and 60 dpi. **Fig. 3.** Content of lipids in the germ balls of sporocyst and redia, 20, 40 and 60 dpi. **Fig. 4.** Content of lipids in the pharynx and sucker of redia and cystogenous gland cells of cercaria, 40 and 60 dpi

mulated lipids in the tegument, oral and ventral suckers, and in the intestine. Much fewer lipid droplets were observed in the cystogenous gland cells.

Cytophotometric assays showed that in the tegument of the sporocyst and redia the amount of lipid is small, i.e. 20–21 WU in sporocysts and 19–20 WU in rediae, the difference not being significant ($p>0.05$). On the other hand, the lipid content of the cercarial tegument is very large, i.e. 92–93 WU and statistically highly significant ($p<0.001$) compared to the redia and sporocyst (Table II, Fig. 2). The mean lipid content of germ balls of sporocysts was 58–60 WU, and for rediae 59–62 WU, (Table II, Fig. 3) and these differences were not statistically significant ($p>0.05$). Lipid values for the redial pharynx and cercarial suckers were 96–97 WU and 97–99 WU, respectively (Table II, Fig. 4), and these differences are not statistically significant ($p>0.05$). Cytophotometric assays of lipids in the cystogenous gland cells of the cercaria revealed a lipid content of only 20–21 WU (Table II, Fig. 4) an amount considerably lower than that found in the tegument and suckers of the cercaria ($p<0.01$).

Discussion

Both the histochemical and cytophotometric assays show that the digestive glands of those snails parasitised by sporocysts, rediae, and cercariae of *F. hepatica* are reduced in the amount of their neutral (storage) lipids. It has been shown previously (Humiczewska and Taracha 1984, 1988; Humiczewska 1991) that carbohydrate levels are also greatly reduced during a parasitic infection, a finding corroborated by other authors (Ginetsinskaya and Dobrovolskiy 1966, James and Bowers 1967, Becker 1980, Yavorskiy 1989, Pokora *et al*. 1993, Klobucar *et al*. 1997). The largest reduction in *G. truncatula* carbohydrates occurred after 40 dpi, the carbohydrates undergoing a little restitution after 60 days (Humiczewska and Taracha

1988). Lipids, however, were quantitatively reduced as early as after 20 days, no restitution being noticeable (Table I). It follows, then, that reserve lipids are mobilised relatively early, when substantial amounts of glycogen are still present. It is widely known that parasites obtain energy predominantly by catabolism of carbohydrates (Erasmus 1972, Barrett 1981, Smyth and Halton 1983), so they deplete the host's carbohydrate reserves at the onset of infection, while the snails draw energy by decomposing lipids. This is very plausible, particularly in view of the fact that lipids are virtually the sole energy source in numerous fasting or hibernating animals. A parasitic infection confronts an organism with a situation resembling that of fasting (Duncan *et al*. 1987).

At the time when the neutral lipid resources are depleted in the digestive glands of the trematode-infected snails, considerable amounts of lipids are detected in the parasites (Figs 2–4). It is worth noticing that there is a large concentration of lipids in tegument and suckers of cercaria and pharynx of redia and also in germ balls of sporocysts and cercariae, i.e. in metabolically active tissues. It could be suggested that lipids disintegrate here; it is supported by the fact that the amount of lipids does not increases as infection proceeds. In germ balls and pharynx of rediae 40 dpi, for example, the increase in lipid is slight in comparison with rediae of 20 dpi, and this is statistically insignificant ($p>0.05$). It is generally believed that parasites, particularly adult ones, are incapable of utilising lipids as a source of energy (Ginetsinskaya and Dobrovolskiy 1966, Reznik 1968), rather that lipids are the end product of carbohydrate catabolism. Some authors claim that the problem concerns a general loss of various lipids such as cholesterol, phospholipids, rather than secretion of specific end products of lipid decomposition. *F. hepatica* and some other trematode species were found to secrete, via the excretory system cholesterol and its esters, triacylglycerols, free fatty acids, and phospholipids (Reznik 1968, Voogt 1972). There is at present no logical explanation as to why these parasites accumulate lipids which they are incapable of using.

The apparent inability of these parasites to degrade lipids is questioned by some authors. For example, Mendlowitz *et al*. (1960) and Erasmus (1972) concluded from the presence in those parasites of lipase and esterase, i.e. enzymes participating in lipid degradation, that neutral lipids could constitute energy reserves. Moreover, numerous authors support the view that adult trematodes transfer lipids to the forming eggs, parthenogenetic forms transfer lipids to the developing embryonic spheres (Becker 1980, Smyth and Halton 1988, Yavorskiy 1989).

Lipid metabolism was unequivocally, albeit indirectly, demonstrated in larval stages (Voogt 1972, Humiczewska and Taracha 1984, Esteves *et al*. 1997). Developmental stages of such parasites as *F. hepatica* and *Schistosoma mansoni* as well as larvae of certain nematodes showed the presence of enzymes participating in lipid degradation. For example, cercariae of *S. mansoni* as well as sporocysts, rediae, and cercariae of *F. hepatica* were found to contain lipases and nonspecific esterases (Mendlowitz *et al*. 1960, Cheng 1965, Frayha and Smyth 1984, Humiczewska 1991, Humiczewska and Rajski 2005). In addition, Žd'ařská (1972) detected nonspecific esterases in sporocysts and cercariae of *Dicrocoelium lanceolatum*, while Humiczewska and Taracha (1988), Humiczewska (1991), Humiczewska and Rajski (2004) found beta-hydroxybutyric and acetyl-CoA dehydrogenases in the *F. hepatica* stages parasitising snails. The presence of active lipid degrading enzymes in sporocysts, rediae, and cercariae supports the view that in developmental stages of *F. hepatica* lipids are normally used as an energy source rather than constituting ballast substances.

In conclusion, all the developmental stages of *F. hepatica*, i.e. sporocysts, rediae and cercariae were observed to accumulate lipid deposits. The presence of the largest amounts of lipids in larval stages of *F. hepatica* in those tissues known to be active metabolically (tegument, embryonic spheres) suggests utilisation of lipids as an energy source. The loss of lipids in the digestive gland of the parasite infected snails is an provides evidence that the snails mobilise energy resources accumulated in that form to compensate for the loss of carbohydrates in these parasites.

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