FOLIA PARASITOLOGICA 42: 81–89, 1995

Carbohydrate and amino acid metabolism in *Giardia*: a review

E. L. Jarroll[1] and **T. A. Paget**[2]

[1]Department of Biology, Cleveland State University, 1983 E. 24th Street, Cleveland, OH 44115 USA;

[2]Department of Biology, Birkbeck College, University of London, Malet Street, London WCIE 7HX UK

Key words: *Giardia*, biochemistry, metabolism, carbohydrates, amino acids

Giardia intestinalis [syn. *G. lamblia*] was first cultured axenically *in vitro* about 20 years ago by Meyer (1976). This milestone has made possible studies of *Giardia*'s biochemistry and metabolism that could not have been accomplished without it. The earliest reports on *Giardia* carbohydrate and energy metabolism suggested glucose as the primary energy source for these parasitic protozoa. However, controversy has arisen recently regarding whether or not amino acids may also play a major role in *Giardia*'s energy metabolism. While this review will examine the current state of knowledge about carbohydrate and amino acid biochemistry and metabolism in *Giardia*, and will attempt to identify areas where gaps in our knowledge exist, it is important to realize that since 1976 there have been many changes in the composition of the *in vitro* growth medium used for *Giardia* cultivation. Although these changes have greatly improved the reproducibility of culture growth, we still lack, and would benefit greatly from, a chemically defined medium for growing *Giardia* trophozoites *in vitro*. Thus, the reader should be aware that the metabolic labelling studies discussed in this paper were performed in a chemically undefined medium which includes serum and often bile in its list of components.

Another milestone which has occurred in the study of *Giardia* biochemistry and metabolism has been the development of *in vitro* methods of encystment (Gillin et al. 1987, Schupp et al. 1988). With the ability to stimulate *in vitro* encystment has come the opportunity to examine aspects of metabolism while the trophozoites are undergoing the process. Indeed, aspects of metabolism under encystment conditions have already proven quite interesting. For example, recent studies suggest that the stimulation of oxygen uptake by exogenous glucose and the killing effect of metronidazole, both of which occur in non-encysting trophozoites, cease within a few hours after encystment is induced (unpublished results). Also with encystment comes the induction of an entire carbohydrate synthesizing pathway which is quiescent in non-encysting trophozoites (Macechko et al. 1992).

Carbohydrates

Determination of the sugar composition of *Giardia* has been based on histochemical (Hill et al. 1981, Ward et al. 1985, 1988) and biochemical (Jarroll et al. 1989, Ortega-Barria et al. 1990, Manning et al. 1992) analyses. In general, those based on histochemical analyses have been less reliable than those based on biochemical analyses and thus will be omitted here. Three gas chromatographic [GC] and mass spectrometric [MS] analyses of total carbohydrates have been reported: one for *G. intestinalis* trophozoites and *in vivo* and *in vitro* derived cysts (Jarroll et al. 1989); one for *G. intestinalis* trophozoite membranes and glycoproteins which were obtained either from encysting or non-encysting trophozoites (Ortega-Barria et al. 1990); and one for *in vivo* derived *G. muris* cysts (Manning et al. 1992). In these analyses, sugars were derivatized following acid hydrolysis by either trimethylsilane or alditol acetate prior to quantitation. For ease of comparison, values given in original articles as nmoles 10^{-6} trophozoites or cysts have been converted to nmoles (100 μg protein)$^{-1}$.

A caveat exists for sugar analyses of *in vivo* derived *Giardia cysts:* while the method for separating them from faecal material yields preparations virtually free of biochemically detectable bacterial contamination (Jarroll et al. 1989), the possibility exists that small amounts of such contamination may remain. Thus, the quantitation of sugars which are in low concentration and the presence of these sugars in *in vivo* derived cysts must be considered tentative until they are confirmed in studies using axenically cultured cells. Caveats must

[1]Author to whom all correspondence should be directed.

also be placed on the carbohydrate analyses of sugars associated with membranes and those of affinity-purified (using wheat germ agglutinin [WGA] bound to sepharose) glycoproteins of axenically grown *G. intestinalis* (Ortega-Barria et al. 1990). First, the membrane fractions were prepared following sonication and centrifugation without the benefit of membrane markers, proteins were protected from the action of known *Giardia* thiol proteinases only by PMSF (phenylmethylsulfonyl fluoride) which is probably not effective (Hare et al. 1989), and extracted proteins represent only those that were soluble in CHAPS (Ortega-Barria et al. 1990). A summary of selected neutral and amino sugar concentrations is shown in Table 1.

Neutral sugars. Glucose is the principal neutral sugar detected in the total sugars from non-encysting *G. intestinalis* trophozoites (Table 1). *G. intestinalis* type [MR4] *in vitro* derived cysts (not shown in Table 1) had 2,667 nmoles glucose (100 µg protein)$^{-1}$ (Jarroll et al. 1989) which is 2- to 5-fold greater than the glucose levels in either non-encysting trophozoites or *in vivo* derived cysts. Lysing the non-encysting trophozoites in the cultures used to generate *in vitro* derived cysts could have left residual glucose probably as endogenous glycogen-like reserve material (Manning et al. 1992, Edwards et al. 1994) in the pellets of cysts collected for analysis. This would artificially raise the amount of glucose relative to the number of cysts counted (or amount of protein). Further investigation will be required to ascertain whether trophozoites preparing to encyst actually accumulate more glucose than those that are not encysting; unpublished data presented later in this review argue against this possibility. Glucose was also the most abundant neutral sugar detected in *G. intestinalis* trophozoite [strain WB] membranes. The fact that glucose is the principal sugar in *Giardia* cysts and trophozoites is not unexpected since, to date, it is still the only sugar shown to be used by trophozoites for energy metabolism (Lindmark 1980).

The second most commonly encountered neutral sugar in *Giardia* cysts and trophozoites is galactose (Table 1). However, it, plus the other non-glucose neutral sugars combined, represent only about 14-17% of the total sugar in *Giardia* cysts.

Mannose (Table 1) was not detected in *G. intestinalis* total sugars (Jarroll et al. 1989), but was detected in sugars from trophozoite membranes and in affinity purified trophozoite glycoproteins (Ortega-Barria et al. 1990). Mannose was also undetected in total sugars from *G. intestinalis* cysts (Jarroll et al. 1989) or encysting trophozoite membranes (Ortega-Barria et al. 1990), but it was detected in total sugars from *G. muris* cysts (Manning et al. 1992). Mannose was present in the affinity purified glycoproteins from encysting *G. intestinalis* (Ortega-Barria et al. 1990). Interestingly, Macechko et al. (1992) were unable to detect incorporation of labelled galactose or mannose into cellular sugars of *Giardia* trophozoites.

Ribose has been detected in the total sugars (Table 1) from *G. intestinalis* trophozoites (Jarroll et al. 1989) and in both *G. intestinalis* and *G. muris* cysts (Jarroll et al. 1989, Manning et al. 1992). Ribose was not reported in total membrane sugars or glycoproteins (Ortega-Barria et al. 1990) suggesting that the ribose may be associated with nucleic acids remaining in the cyst wall preparations following the various chemical treatments.

Fucose has been reported (Table 1) in *G. intestinalis* trophozoites and encysting trophozoite membrane sugars at very low levels (Ortega-Barria et al. 1990). A trace amount of fucose was reported in *G. muris* cyst preparations following treatment of the cysts by SDS (Manning et al. 1992). Xylose, arabinose, and rhamnose have also been reported in *G. muris* cyst samples in small amounts (Table 1) (Manning et al. 1992). These sugars must be regarded as possible contaminants (see above) until their presence has been confirmed in axenically cultured trophozoites or *in vitro* derived cysts.

Amino sugars. It is possible that amino sugars could exist in these cells in either the N-acetylated or non-acetylated form. Because it has not yet been determined which form is present in *Giardia* and because following acid hydrolysis the sugars are detected only in the non-acetylated form (unless specifically re-acetylated prior to analysis), only the name of the non-acetylated amino sugars will be used when describing the analyses in this review.

Glucosamine (Table 1) was not detected in the total sugars from *G. intestinalis* trophozoites, *in vivo* derived cysts or *in vitro* derived cysts (Jarroll et al. 1989), but it was detected in the membrane sugars of non-encysting *G. intestinalis* trophozoites and in affinity purified glycoproteins (Ortega-Barria et al. 1990). Glucosamine was detected in the membrane sugars, in the affinity purified glycoproteins (Ortega-Barria et al. 1990) of *G. intestinalis*, and glucosamine was detected in intact *G. muris* cysts in small quantity (Manning et al. 1992).

Galactosamine (Table 1) has been detected in the total sugars of *G. intestinalis in vivo* derived and *in vitro* derived cysts (Jarroll et al. 1989), and in the membrane sugars of encysting *G. intestinalis* trophozoites (Ortega-Barria et al. 1990). In *G. muris* cysts, galactosamine is nearly equivalent to glucose in amount, and the second most abundant sugar in *G. intestinalis* cysts. Galactosamine was not detected in either total, membrane or affinity-purified glycoprotein sugars from non-encysting *G. intestinalis* trophozoites (Jarroll et al. 1989, Ortega-Barria et al. 1990). The extracts used for the membrane and glycoprotein analyses (Ortega-Barria

Table 1. Summary of neutral and amino sugars reported from *Giardia* cysts and trophozoites.

	Cysts		Trophozoites		
Sugar	*G. intestinalis**	*G. muris***	*G. intestinalis**	Membranes***	Glycoproteins****
Glucose	707	893	597	6.1 [45.6]	46.2 [37.6]
Galactose	123	85	–	1.5 [0]	25.6 [6.4]
Mannose	–	32	–	1.0 [14]	– [3.6]
Ribose	62	195	12	–	–
Fucose	–	tr	–	0.5 [0]	5.8 [0]
Glucosamine	–	30	–	0.5 [21.2]	3.0 [15.2]
Galactosamine	195	884	–	–	3.4 [0]
Sialic acid	–	–	–	1.6 [0]	16.0 [0]

* These values are in nmoles (100 g protein) $^{-1}$ and are conversions of the data from Jarroll et al. (1989).

** These values are in nmoles (100 g protein)$^{-1}$ and are conversions of the data from Manning et al. (1992).

***These values are in nmoles (100 g protein)$^{-1}$ from non-encysting trophozoites and are taken from Ortega-Barria et al. 1990.

Values in [ ] are from encysting cell membranes and glycoproteins.

tr = trace

– = not detected.

et al. 1990) were obtained from encysting cells collected after 24 hrs in encystment medium and would have excluded completely formed cysts and encystment specific vesicles ESVs (Faubert et al. 1991) likely to be present in trophozoites undergoing transition to fully formed cysts. ESVs contain material (amino acids and carbohydrates especially galactosamine) destined to form portions of the mature *Giardia* cyst wall. Much of this vesicular material is detergent (sodium dodecyl sulfate, SDS) insoluble (unpublished observation), and thus, was likely excluded from these analyses.

Sialic acid (NANA) was not detected in the total sugar analyses of either *G. intestinalis* trophozoites, *G. intestinalis* cysts or *G. muris* cysts (Jarroll et al. 1989, Manning et al. 1992). NANA was reported in the membrane of *G. intestinalis* trophozoites and in affinity purified trophozoite glycoproteins (Ortega-Barria et al. 1990).

Polysaccharides. It has long been assumed that *Giardia* store glucose as glycogen, but this assumption has never been proven biochemically. Currently, it is clear that glucose is the major sugar component of *Giardia* trophozoites and that in cysts it is nearly equal in quantity to galactosamine (see above). Dutta (1965) reported that periodic acid Schiff reaction [PAS] staining of *G. intestinalis* cysts was abolished following treatment by salivary amylase or malt diastase. Manning et al. (1992) demonstrated that the interior of intact and SDS-treated (interior proteins and lipids removed) *G. muris* cysts stain positively with PAS while SDS-treated *G. muris* cysts subsequently treated with amylo-glucosidase failed to stain with PAS. Furthermore, the sugar analyses of intact, SDS-treated and SDS amylo--glucosidase-treated *G. muris* cysts support this observation. The level of glucose in intact and SDS-treated *G. muris* cysts ranged from ca. 739–924 nmoles (100 µg protein)$^{-1}$ cysts (Manning et al. 1992) while that in SDS, amyloglucosidase-treated cysts drop to 23 nmoles (100 µg protein)$^{-1}$ cysts. Edwards et al. (1994) reported ^{13}C-nuclear magnetic resonance (NMR) signals for a glycogen-like polysaccharide in perchloric acid extracts of trophozoites. These findings suggest that glucose is stored as glycogen in *Giardia*, but there is no confirmatory biochemical evidence that the endogenous glucose storage substance has, in fact, the proper glycosidic linkages to be designated as glycogen. Furthermore, there are no reports of enzymes that catalyze glycogen synthesis or degradation in *Giardia*.

Despite earlier studies to the contrary (Filice 1952, Dutta 1965), chitin [ß1—>4 homopolymer of GlcNAc made by the action of chitin synthetase] was reported as a major *Giardia* cyst wall component based on lectin binding studies (Ward et al. 1985). However, based on GC/MS analyses and lectin binding studies, the cyst wall of *Giardia* is composed mainly of galactosamine [GalN, probably as GalNAc based on lectin binding affinity] in an as yet unknown configuration.

Carbon and Energy Metabolism in *Giardia*

Up until the mid 1980's much of our knowledge of the catabolic carbohydrate metabolism of *Giardia* was based on the works of Lindmark (1980), Weinbach et

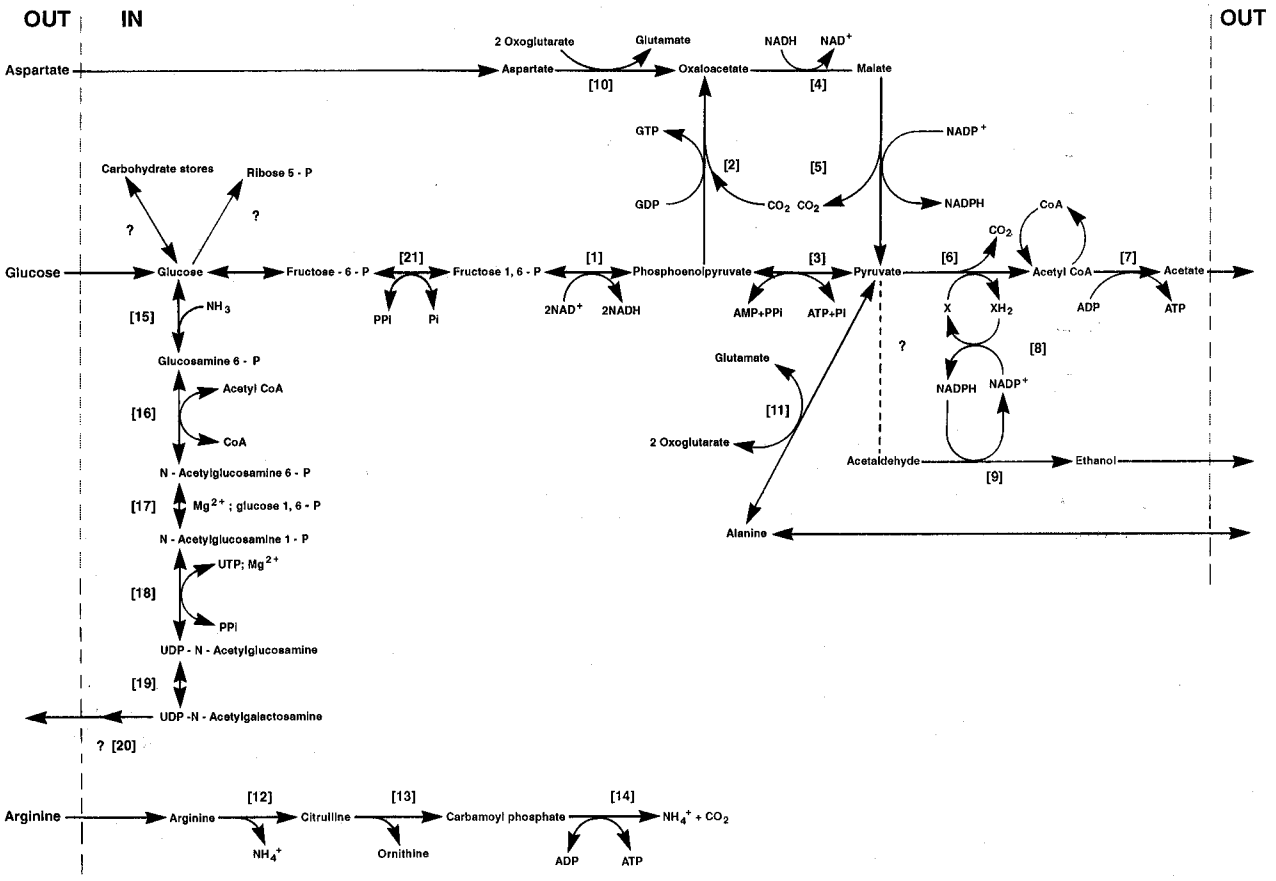

Fig. 1. A summary of metabolic pathways present in *Giardia*. Glucose, alanine, arginine and aspartate are shown as carbon and energy sources. PPi-dependent phosphofructokinase [1], enzymes of the presumed Embden Meyerhof Parnas pathway [2]; phosphoenolpyruvate carboxykinase (GDP) [3]; malate dehydrogenase (NAD) [4]; malate dehydrogenase (decarboxylating, NADP) [5]; pyruvate phosphate dikinase [6]; pyruvate: ferredoxin oxidoreductase (PFOR) [7]; acetyl-CoA synthetase (ADP) [8]; NAD[P]H oxidase [9]; alcohol dehydrogenase (NADP) [10]; alanine: 2-oxoglutarate transaminase [11]; aspartate: 2-oxoglutarate transaminase [12]; arginine deaminase [13]; catabolic ornithine transcarbamoylase [14]; carbamate kinase [15]; glucosamine 6-phosphate isomerase [16]; glucosamine 6-phosphate N-acetylase [17]; glucosamine 6-phosphate mutase [18]; UDP-N-acetylglucosamine pyrophosphorylase [19]; UDP-N-acetylglucosamine 4'-epimerase [20]; "cyst wall synthetase" activity [21].

al. (1980) and Jarroll et al. (1981). These pioneering studies indicated that this "anaerobic" protozoan uses glucose as its major carbon source. Among the reasons for this was that 1) no other substrates tested affected respiration, 2) many of the enzymes associated with glycolysis were demonstrated, and 3) ethanol, acetate and carbon dioxide were detected as the major end products of glycolysis. A functional TCA cycle was not detected in this organism and it was suggested that substrate level phosphorylation is the mechanism for energy generation in *Giardia*. Besides glycolysis, the components of an anaerobic electron transport system involving flavins, iron-sulphur proteins and an enzyme complex similar to pyruvate : ferredoxin oxidoreductase [PFOR] have been reported in *Giardia*. Recent studies (Ellis et al. 1993, Townson et al. 1994b) indicate that this enzyme is PFOR and that some components of this electron transport system are membrane associated. Questions raised in earlier studies such as: How is the ethanol formed metabolically?; What is the ultimate fate of the electrons transported by the anaerobic electron transport system?; and How does this "anaerobic" organism cope with oxygen? are still incompletely understood. It is important that these aspects of metabolism are studied in depth as this information could aid in our understanding of metronidazole's mode of action.

Metronidazole is commonly used for the treatment of giardiasis (Boreham 1994); however, its mode of action

in *Giardia* is still unknown. In *Trichomonas vaginalis*, metronidazole is reductively activated by hydro- genosomal electron transport. In whole cells, the metronidazole free radical anion has been detected (Lloyd and Pedersen 1985), and it is thought that the anion is responsible for cell death by causing damage to membranes and to DNA. Metronidazole resistance in *T. vaginalis* correlates with defects in the oxygen scavenging capacity of NADH oxidase (Yarlett et al. 1988). It is also believed that elevated levels of intracellular oxygen interfere with the reduction and activation of metronidazole. It is likely that the mode of action of metronidazole in *Giardia* is similar to that in *T. vaginalis* though studies to date have been inconclusive. Metronidazole inhibits oxygen uptake and NADH oxidase activity in *Giardia* trophozoites by 80 and 50%, respectively, however, the concentrations required to achieve these inhibitions were significantly higher than those required to inhibit cell growth (Paget et al. 1993, See et al. 1994). The biochemical basis of metronidazole resistance has also been studied in *Giardia* (Boreham et al. 1993, Ellis et al. 1993) and it appears that the mechanism is different from that in *T. vaginalis*.

A functional pentose phosphate pathway has been reported in *Giardia* based on the evolution of CO_2 from [1-^{14}C] glucose (Jarroll et al. 1981), but these protozoans appear unable to synthesize purines and pyrimidines *de novo*. *Giardia* trophozoites are, however, avid scavengers of purines and pyrimidines (Jarroll and Lindmark 1990). Furthermore, *Giardia* trophozoites are lipid auxotrophs apparently salvaging lipids from intestinal sources (Jarroll and Lindmark 1990). These observations, plausible because *Giardia* trophozoites inhabit an environment rich in biosynthetic precursors, suggest that *Giardia*'s energy requirement for biosynthesis is also reduced. Such a reduction would seem beneficial to a fermentative parasite. For a more detailed description of these studies there are several reviews on this area (Jarroll and Lindmark 1990, Muller 1991).

Amino Acids as Energy and Carbon Sources

By the end of the 1980's, techniques such as NMR and MS were being routinely applied to the study of parasites. The first major discovery arising from their use was that alanine represents a major end product of *Giardia* metabolism. With the detection of an alanine aminotransferase came the suggestion of a new pathway in *Giardia,* and the first description of the *de novo* synthesis of an amino acid in *Giardia* (Mendis et al. 1992). Mendis et al. (1992) showed that aspartate is metabolized via alanine-2-oxoglutarate transaminase to oxaloacetate and glutamate (Fig. 1). Oxaloacetate is then converted to malate and finally to pyruvate with the formation of CO_2, NADPH and NAD^+. Additionally, this group has also shown that the formation of alanine from pyruvate is reversible and they suggested a metabolic map for *Giardia* expanded from that published by Lindmark (1980).

Schofield et al. (1990, 1992) showed that arginine can be catabolized by the arginine dihydrolase pathway in *Giardia* (Fig. 1) and that the pathways of glucose and arginine catabolism are not directly connected. They also suggested that the flux through the arginine dihydrolase pathway indicated that the potential yield of ATP by this route was 7-8 fold greater than that from glucose. By implication, it would seem that arginine may be the major bioenergetic substrate for this organism. However, Mendis (1994) stated that the rates of CO_2 liberation are probably more indicative of pentose phosphate pathway activity than of glycolytic pathway activity as suggested since [1-^{14}C] glucose was used as their glucose source.

The importance of glucose as a source of carbon was also questioned by Schofield et al. (1991). They showed that trophozoites can grow in low levels of glucose (2-3 mM) as opposed to those typically found in growth medium (50 mM). Growth rates in medium with 2–3 mM glucose were, however, only 50% of those in medium with the normal 50 mM glucose. These workers also noted that at 2–3 mM glucose the profile of end products was modified. During growth at 50 mM glucose, *Giardia* produces mainly alanine and ethanol with some acetate; however, at 2–3 mM glucose the amount of end products formed is reduced and the major products are alanine and acetate with some ethanol. These observations, while indicative that amino acids are another source of energy and carbon for *Giardia*, do not show conclusively that glucose is less important than was previously thought, especially since trophozoites grow at a faster rate in the presence of 50 mM glucose and since the data on flux are inconclusive.

The recent observations of pyrophosphate dependent phosphofructokinase (PPi-PFK) and pyruvate phosphate dikinase in *Giardia* (Mertens 1990, Hrdý et al. 1993) suggest an alteration in the potential for ATP synthesis from glycolysis that would have been predicted previously. Pyruvate phosphate dikinase in combination with adenylate kinase, converts phosphoenolpyruvate (PEP) to pyruvate with the formation of two ATP molecules as follows:
Adenylate kinase catalyzes the conversion of ADP to ATP and AMP

$$2\ ADP \longrightarrow ATP + AMP$$

while pyruvate phosphate dikinase catalyzes the conversion of AMP to ATP

$$PEP + AMP + Pi \longrightarrow ATP + Pi + pyruvate$$

Thus, the modified glycolytic pathway in *Giardia* can theoretically yield 5 moles of ATP per mole of glucose; however, Mertens (1993) suggests that in the presence of oxygen, this yield may increase to 7 with the possibility of one extra mole of ATP being generated by the conversion of each pyruvate to acetate via a mechanism that is as yet unclear. Rationale for the use of PPi as an energy substrate in other amitochondrial anaerobes has been suggested by others (Hrdý et al. 1993, Mertens 1993). The increase in glycolytic capacity for ATP synthesis is a major advantage as may be the ability to synthesize glucose from amino acids.

Giardia in situ

The intestinal tract, the normal habitat for *Giardia* trophozoites, has been long thought of as anaerobic; however, it is now clear from direct and indirect measurements that oxygen exists in this environment at levels which fluctuate between 0–60 μM (Atkinson 1980). Trophozoites can survive for several hours in the presence of oxygen concentrations less than 50 μM suggesting that trophozoites of *G. intestinalis* and *G. muris* are probably aerotolerant; trophozoites maintained in buffer with glucose are sensitive to oxygen concentrations greater than 80 μM (Paget et al. 1989, 1993). The effect of oxygen on fermentation pathways in *Giardia* is, thus, another variable that must be taken into account. Oxygen alters the ratios and amounts of end products formed by *Giardia* trophozoites in buffer. For example in the presence of 3 μM dissolved O_2, acetate and carbon dioxide predominate, ethanol concentrations are reduced to anaerobic levels, and the concentration of alanine falls to barely detectable levels (Paget et al. 1993). This switch may be in response to changes in cellular redox state or it may indicate that the catabolism of glucose to acetate is more energetically favourable (Mertens 1993). It has not yet been possible to determine if *Giardia* is microaerophilic. Controlled growth under defined and controlled oxygen and carbon dioxide concentrations (Paget and Lloyd 1990) such as those used to show that *Trichomonas vaginalis* is microaerophilic have not been possible since *Giardia* requires cysteine for growth. Cysteine rapidly reacts with oxygen to form a variety of radical species (Paget and Lloyd 1988). Hence, the cultivation of *Giardia* under controlled oxygen conditions is difficult and the concomitant generation of radicals could produce anomalous results.

Cyst Metabolism

All of the metabolic studies discussed previously relate to the trophozoite form of *Giardia*, but this protozoan also exhibits the infective cyst form. Work on cysts has focused mainly on determining the viability of, the efficacy of disinfectants on, and the survival of cysts in the environment. At 37°C, cysts will only remain viable for a few days, while at 4°C, this period increases to 1–2 months (Bingham et al. 1979). This sensitivity to temperature suggested that cysts are metabolically active. Lindmark and Miller (1987) showed that *G. intestinalis* and *G. muris* cysts contained the same glucose catabolizing enzymes as, and at levels comparable to, those of metabolically active *G. intestinalis* trophozoites. Cysts of *G. intestinalis* and *G. muris* show rates of oxygen uptake approximately 10–15% that of trophozoites. Oxygen uptake by cysts is stimulated by ethanol, but not by any of the carbohydrates or TCA intermediates tested (Paget et al. 1989, 1993b). Thus, *Giardia* cysts are not true cryptobiotic forms since they are apparently metabolically active presumably catabolizing a glucose-containing carbohydrate storage product (Manning et al. 1992, Edwards et al. 1994), and their oxygen uptake exhibits inhibitor sensitivity (Paget et al. 1989, 1993b). The sensitivity of cyst oxygen uptake to inhibitors, with the exception of metronidazole, is similar to that observed for trophozoites.

Giardia cysts are resistant to metronidazole which is quite toxic to the trophozoite form of the organism. The mechanism of resistance, though not yet determined, could be due to metabolic insufficiency, reduced rate of oxygen consumption raising intracellular oxygen concentrations which may compete with the PFOR complex for electrons, or the impermeability of cysts to metronidazole.

The *in vitro* encystment process, initiated by the addition of 10% bile (w/v) to growth medium, produces cysts which can first be observed after 16–24 hrs. However, the percent encystment increases with time and typically, encystment at 72 hrs varies between 30–50%. Interestingly, other significant metabolic changes occur very rapidly over the course of the first 24 hrs and typically after 8–14 hrs (Macechko et al. 1992, Paget et al. 1994). For example, oxygen uptake by encysting cells is apparently unaffected by metronidazole and exogenous glucose after 12 hrs into the encystment process. After 72 hrs, the rate of oxygen uptake for the encysting cells is similar to that for mature *in vivo* derived cysts (Paget et al. 1994). Furthermore, recent studies (unpublished results) indicate that glucose uptake by encysting trophozoites appears to cease 12–15 hrs after the initiation of encystment. During encystment, glucose, probably from endogenous reserves, is used to synthesize UDP-GalNAc by an inducible pathway of enzymes (Fig. 1) (Macechko et al. 1992). The GalNAc formed is used to synthesize a large portion of the *Giardia* outer filamentous cyst wall (see above). For this synthesis, glucose is converted to fructose 6-phosphate most likely via the normal glycolytic route. Fructose 6-phosphate and ammonia are converted to glucosamine 6-phosphate

by the action of glucosamine 6-phosphate isomerase. This enzyme has been purified and characterized from encysting *Giardia* as a single enzyme which is reversible (Steimle and Jarroll 1994). Glucosamine 6-phosphate is then acetylated to GlcNAc 6-phosphate by the action of glucosamine 6-phosphate N-acetylase. The N-acetylated product is converted to GlcNAc 1-phosphate by the action of glucosamine 6-phosphate mutase. UDP-GlcNAc pyrophosphorylase in the presence of GlcNAc 1-phosphate and UTP forms UDP-GlcNAc which is then epimerized to UDP-GalNAc by UDP-GlcNAc 4'-epimerase (Macechko et al. 1992). At this point, it is possible to detect a previously unknown enzyme activity (tentatively called "cyst wall synthetase", CWS) which apparently behaves in a manner similar to that of chitin synthetase except that it exhibits remarkable specificity for UDP-GalNAc (Karr et al. 1994). The CWS activity fixes UDP-GalNAc into an ethanol precipitable, SDS-insoluble, amylase-resistant product which does not migrate in SDS-polyacrylamide gel electrophoresis. This ethanol precipitate from the CWS assay behaves in the same fashion that the cyst wall filaments from mature cysts behave (Jarroll et al. 1989, Manning et al. 1992). Because the structural nature of the GalN in the *Giardia* cyst wall and in the product of this UDP-GalNAc fixing enzyme are not known, it is impossible to describe the enzyme's activity further at this time.

While the mechanism of oxygen reduction in *Giardia* and its stoichiometry are unknown, the level of oxygen uptake in cysts represents a substantial rate of substrate utilization. If the glucose concentration in trophozoites is roughly 130 nmoles 10^{-6} trophozoites (Jarroll et al. 1989), that in cysts is 64 nmoles 10^{-6} cysts (Jarroll et al. 1989) and if the rate of oxygen uptake in cysts is 0.5–1 nmoles oxygen 10^{-6} cysts min^{-1} (Paget et al. 1993), then it would seem conceivable that the endogenous carbohydrate store may be insufficient to supply both cyst wall material and metabolic activity of cysts over prolonged periods. Do encysting trophozoites, therefore, synthesize the cyst wall from other exogenous source such as amino acids or do they shift their energy metabolism away from glucose and toward amino acids so that the glucose can serve as a source for GalNAc?

Concluding Remarks

Our present knowledge of the pathways of glucose, alanine and arginine metabolism is depicted in Fig. 1. From the data available, it is impossible to say which, if any, of the potential substrates is most favoured catabolically by *Giardia*. To answer this question, study of the metabolic flux of glucose using [6-^{14}C] gluco and [3,4-^{14}C] glucose would be required as well as a re-evaluation of the ATP-producing potential of the glycolytic pathway in *Giardia*. These studies should be performed under conditions which mimic the *in situ* environment of approximately 5 mM CO_2 and $O_2 < 60$ µM. Work on the expression of catabolic genes such as glutamate dehydrogenase (Yee and Dennis 1994) may also prove a useful adjunct to metabolic studies on substrate use. With our present understanding of the catabolic metabolism of *Giardia*, it is not possible to define absolutely the roles played by carbohydrates or amino acids. However, it seems likely that an organism such as *Giardia* would be opportunistic in its use of substrates, since its *in situ* environment is rich in a variety of potential substrates, and thus the idea of a "major substrate" may not be realistic.

Our understanding of *Giardia*'s ability to detoxify oxygen is at present hampered by our inability to grow this organism in a more defined medium without excess cysteine. The recent work on *Giardia*'s amino acid metabolism, PPi-linked enzymes and membrane-associated electron transport system has highlighted the similarities and differences among *Giardia*, *Entamoeba* and *Trichomonas* (Lloyd and Paget 1991 ,Mertens 1991, Muller 1991, Ellis et al. 1993); such comparisons also emphasize evolutionary diversity among these organisms.

We have been able to extend the scope of biochemical and metabolic studies to *Giardia* cysts and to the encystment process. Further work is needed on the cysts and on the encystment process since cysts and their formation are integral to the transmission of giardiasis. Thus, attacking the formation of cysts may represent a potentially useful approach for chemotherapeutic attack on giardiasis. It is important to remember that *Giardia* is a very common human pathogen and PFOR is the site of activation for metronidazole, a drug still commonly used in the treatment of giardiasis. Resistance to and the limits imposed on the use of metronidazole makes the study of catabolic metabolism very important. Of additional importance to chemotherapy may be the targeting of the encystment-induced GalNAc synthesizing pathway recently described in *Giardia*. Targeting this pathway may have more significance if the reported resistance to metronidazole increases (Townson et al. 1994a). Beyond the implications that this GalNAc synthesizing pathway offers for chemotherapy is its importance in understanding, perhaps for the first time in a protozoan, the molecular aspects of a major cytodifferentiation process leading from trophozoite to cyst formation. Even though the study of metabolism in *Giardia* has gone through a renaissance, it is clear that there are many unanswered questions left to explore.

REFERENCES

ATKINSON H. J. 1980: Respiration. In: B. H. Zuckerman (Ed.), Nematodes as Biological Models. Academic Press, London, pp. 101–138.

BINGHAM A., JARROLL E., MEYER E., RADULESCU S. 1979: *Giardia* sp.: physical factors of excystation *in vitro* and excystation vs. eosin exclusion as determinants of viability. Exp. Parasitol. 47: 284–291.

BOREHAM P. 1994: The current status of chemotherapy for giardiasis. In: R. C. A. Thompson, J. A. Reynoldson, and A. J. Lymbery (Eds.), *Giardia*: From Molecules to Disease. CAB International, Oxon, UK, pp. 317–328.

BOREHAM P., UPCROFT J., UPCROFT P. 1991: Biochemical and molecular mechanisms of drug resistance to nitroheterocyclic drugs in *Giardia intestinalis*. In: G. H. Coombs, and M. A. North (Eds), Biochemical Protozoology. Taylor & Francis, London, pp. 594–604.

DUTTA G. 1965: Demonstration of neutral polysaccharides with fluorescence microscopy using acridine orange. Nature 205: 712.

EDWARDS M., GROTE R., PAYNE K., O'SULLIVAN W. 1994: ^{31}P and ^{13}C NMR studies of *Giardia intestinalis*. In: R. C. A. Thompson, J. A. Reynoldson and A. J. Lymbery (Eds.), *Giardia*: From Molecules to Disease. CAB International, Oxon, UK, pp. 185–186.

ELLIS J. E., WILLIAMS R., COLE D., CAMMACK R., LLOYD D. 1993: Electron transport components of the parasitic protozoan *Giardia lamblia*. FEBS Letts. 325: 196–200.

FAUBERT G., REINER D., GILLIN F. 1991: *Giardia lamblia*: regulation of secretory vesicle formation and loss of ability to reattach during encystation *in vitro*. Exp. Parasitol. 72: 345–354.

FILICE F. 1952: Studies on the cytology and life history of *Giardia* from the laboratory rat. Univ. Calif. Publ. Zool. 57: 53–143.

GILLIN F. D., REINER D., GAULT M., DOUGLAS H., DAS S., WUNDERLICH A., SAUCH J. 1987: Encystation and expression of cyst antigens by *Giardia lamblia in vitro*. Science 235: 1040–1043.

HARE D., JARROLL E., LINDMARK D. 1989: *Giardia lamblia*: characterization of proteinase activity in trophozoites. Exp. Parasitol. 68: 168–175.

HILL D., HEWLETT E., PEARSON R. 1981: Lectin binding by *Giardia lamblia*. Infect. Immunol. 34: 733–738.

HRDÝ I., MERTENS E., NOHÝNKOVÁ E. 1993: *Giardia intestinalis*: detection and characterization of a pyruvate-phosphate dikinase. Exp. Parasitol. 76: 438–441.

JARROLL E., LINDMARK D. 1990: *Giardia* metabolism. In: E. A. Meyer (Ed.), Giardiasis. Elsevier/North Holland, Amsterdam, pp. 61–76.

JARROLL E., MANNING P., LINDMARK D., COGGINS J., ERLANDSEN S. 1989: *Giardia* cyst wall-specific carbohydrate: evidence for the presence of galactosamine. Mol. Biochem. Parasitol. 32: 121–132.

JARROLL E., MULLER P., MEYER E., MORSE S. 1981: Lipid and carbohydrate metabolism of *Giardia lamblia*. Mol. Biochem. Parasitol. 2: 187–196.

KARR C., LINDMARK D., JARROLL E. 1994: Cyst wall synthetase: a novel enzyme induced during *Giardia* cyst wall formation. 47th annual meeting of the Society of Protozoologists. Cleveland State University, Cleveland, OH. June 24–29, 1994.

LINDMARK D. 1980: Energy metabolism of the anaerobic protozoon *Giardia lamblia*. Mol. Biochem. Parasitol. 1: 1–12.

LINDMARK D., MILLER J. 1987: Enzyme activities of *Giardia lamblia* and *Giardia muris* trophozoites and cysts. In: P. Wallis, and B. Hammond (Eds.), Advances in *Giardia* Research. Univ. of Calgary Press, Calgary, pp. 187–189.

LLOYD D., PAGET T. 1991: The effects of environmental factors on the metabolism of *Giardia* and *Trichomonas*. In: G. H. Coombs, and M. A. North (Eds.), Biochemical Protozoology. Taylor & Francis, London, pp. 92–101.

LLOYD D., PEDERSEN J. 1985: Metronidazole radical anion generation *in vivo* in *Trichomonas vaginalis:* oxygen quenching is enhanced in a drug resistant strain. J. Gen. Microbiol. 131: 87–92.

MACECHKO P. T., STEIMLE P., LINDMARK D., ERLANDSEN S., JARROLL E. 1992: Galactosamine-synthesizing enzymes are induced when *Giardia* encyst. Mol. Biochem. Parasitol. 56: 301–310.

MANNING P., ERLANDSEN S., JARROLL E. 1992: Carbohydrate and amino acid analyses of *Giardia muris* cysts. J. Protozool. 39: 290–296.

MEYER E. A. 1976: *Giardia lamblia*: isolation and axenic cultivation. Exp. Parasitol. 39: 101–105.

MENDIS A., THOMPSON R., REYNOLDSON J., ARMSON A., MELONI B., GUNSBERG S. 1992: The uptake and conversion of 1-[$U^{14}C$-] aspartate and L-[$U^{14}C$-] alanine to $^{14}CO_2$ by intact trophozoites of *Giardia duodenalis*. Comp. Biochem. Physiol. 102: 235–239.

MERTENS E. 1990: Occurrence of pyrophosphate: fructose 6-phosphate 1-phosphotransferase in *Giardia intestinalis* trophozoites. Mol. Biochem. Parasitol. 40: 147–149.

MERTENS E. 1991: Pyrophosphate-dependent phosphofructokinase, an anerobic glycolytic enzyme? FEBS Letts. 285: 1–5.

MERTENS E. 1993: ATP versus pyrophosphate - glycolysis revisited in parasitic protists. Parasitol. Today 9: 122-126.

MULLER M. 1991: Energy metabolism of anaerobic protists. In: G. Coombs, and M. North (Eds.), Biochemical Protozoology. Taylor & Francis, London, pp. 80–91.

ORTEGA-BARRIA E., WARD H., EVANS J., PEREIRA M. 1990: N-acetyl-D-glucosamine is present in cysts and trophozoites of *Giardia lamblia* and serves as receptor for wheatgerm agglutinin. Mol. Biochem. Parasitol. 43: 151–166.

PAGET T., JARROLL E., MANNING P., LINDMARK D., LLOYD D. 1989: Respiration in the cysts and trophozoites of *Giardia muris*. J. Gen. Microbiol. 13: 145–154.

PAGET T., LLOYD D. 1988: Effects of reducing agents on the metabolism and morphology of *Trichomonas*

vaginalis C1 NIH. In: D. Lloyd, G. Coombs and T. Paget (Eds.), The Molecular Biology and Biochemistry of Anaerobic Protozoa. Harwood Academic Press, London, pp. 123–136.

PAGET T., LLOYD D. 1990: *Trichomonas vaginalis* requires traces of oxygen and high concentrations of CO_2 for optimum growth. Mol. Biochem. Parasitol. 41: 65–72.

PAGET T., KELLY M., JARROLL E., LINDMARK D., LLOYD D. 1993a: The effects of oxygen on fermentation in *Giardia lamblia*. Mol. Biochem. Parasitol. 57: 65–72.

PAGET T., MANNING P., JARROLL E. 1993b: Oxygen uptake in cysts and trophozoites of *Giardia lamblia*. J. Euk. Microbiol. 40: 246–250.

PAGET T., MANNING P., ERLANDSEN S., JARROLL E. 1994: Metabolic changes during encystation and excystment in *Giardia duodenalis*. In: R. C. A. Thompson, J. A. Reynoldson and A. J. Lymbery (Eds.), *Giardia*: From Molecules to Disease. CAB International, Oxon, UK, pp. 197–198.

SCHOFIELD P., COSTELLO M., EDWARDS M., O'SULLIVAN W. 1990: The arginine dihydrolase pathway is present in *Giardia intestinalis*. Int. J. Parasitol. 20: 697–699.

SCHOFIELD P., EDWARDS M., KRANZ P. 1991: Glucose metabolism in *Giardia intestinalis*. Mol. Biochem. Parasitol. 45: 39–48.

SCHOFIELD P., EDWARDS M., MATHEWS J., WILSON J. 1992: The pathway of arginine catabolism in *Giardia intestinalis*. Mol. Biochem. Parasitol. 51: 29–36.

SCHUPP D., JANUSCHKA M., SHERLOCK L., STIBBS H., MEYER E., BEMRICK W., ERLANDSEN S. 1988: Production of viable *Giardia* cysts *in vitro*: determination by fluorogenic dye staining, excystation, and animal infectivity in the mouse and Mongolian gerbil. Gastroenterology 95: 1–10.

Received 24 November 1994

SEE L., REYNOLDSON J., THOMPSON R., MENDIS A. 1994: NADH oxidase activity in *Giardia* trophozoites: a target for antigiardial agents? In: R. C. A. Thompson, J. A. Reynoldson and A. J. Lymbery (Eds.), *Giardia*: From Molecules to Disease. CAB International, Oxon, UK. pp. 375–376.

STEIMLE P., JARROLL E. 1994: Purification and characterization of glucosamine 6-phosphate isomerase from encysting *Giardia*. 47th annual meeting of the Society of Protozoologists. Cleveland State University, Cleveland, OH. June 24–29, 1994.

TOWNSON S., BOREHAM P., UPCROFT P., UPCROFT J. 1994a: Resistance to the nitroheterocyclic drugs. Acta Tropica 56: 173–194.

TOWNSON S., HANSON G., UPCROFT J., UPCROFT P. 1994b: A purified ferredoxin from *Giardia duodenalis*. Eur. J. Biochem. 220: 439–446.

WARD H., ALROY J., LEV B., KEUSCH G., PEREIRA M. 1985: Identification of chitin as a structural component of *Giardia* cysts. Infect. Immunol. 49: 629–634.

WARD H., ALROY J., LEV B., KEUSCH G., PEREIRA M. 1988: Biology of *Giardia lamblia*. Detection of N-acetyl-D-glucosamine as the only surface saccharide moiety and identification of two distinct subsets of trophozoites by lectin binding. J. Exp. Med. 167: 73–88.

WEINBACH E., CLAGGETT C., KEISTER D., DIAMOND L. 1980: Respiratory metabolism of *Giardia lamblia*. J. Parasitol. 66: 347–350.

YARLETT N., YARLETT N. C., LLOYD D. 1986: Metronidazole-resistant clinical isolates of *Trichomonas vaginalis* have lowered oxygen affinities. Mol. Biochem. Parasitol. 19: 111–116.

YEE J., DENNIS P. 1994: The NADP-dependent glutamate dehydrogenase of *Giardia lamblia* – a study of function, gene structure and expression. Syst. Appl. Microbiol. 16: 759–767.

Accepted 12 April 1995