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Research Article

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Effects of Bile and Bile Salts on Growth and Membrane Lipid Uptake by *Giardia lamblia*

Possible Implications for Pathogenesis of Intestinal Disease

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Abstract

We have shown previously that ox and pig bile accelerate in vitro growth of *Giardia lamblia*. We have now investigated the possible mechanisms by which mammalian biles promote parasite growth. Growth effects of (*a*) ox, pig, guinea pig, and human biles, (*b*) pure bile salts, and (*c*) egg and soybean lecithins were studied in the presence of a lecithin-containing growth medium. Individually, dilute native bile and pure sodium taurocholate (TC), glycocholate (GC), and taurodeoxycholate (TDC) promoted parasite growth; growth was most marked with biles of high phospholipid content, with biles enriched in more hydrophobic bile salts (ox ≃ human > pig > guinea pig) and with micellar concentrations of GC and submicellar concentrations of TC and TDC. By measuring uptake of radiolabeled biliary lipids from bile and bile salt-supplemented growth medium, we showed that the parasite consumed bile lipids, with the rank order lecithin > bile salts. Apparent net uptake of cholesterol was considered to be due to exchange, since net loss of cholesterol from the growth medium was not detected. Although bile and bile salt-stimulated parasite growth was associated with enhanced lecithin uptake, reduction in generation time was observed at low bile and bile salt concentrations when lecithin uptake was similar to bile free controls. Thus, bile salts may stimulate *Giardia* growth initially by a mechanism independent of enhanced membrane phospholipid uptake. However, since *Giardia* has no capacity to synthesize membrane lipid, biliary lecithin may be a major source of phospholipid for growth of this parasite.

Introduction

Giardia lamblia (*Giardia*) is the most common protozoan pathogen of the human intestinal tract and is found worldwide (1). The favored habitat of *Giardia* in humans and animals is the duodenum and proximal jejunum (2, 3). *Giardia* does not readily synthesize its membrane phospholipids *de novo* (4), and may thus be dependent on preformed biliary phospholipid and/or cholesterol, which are abundant in the proximal small intestine. The mechanisms by which this parasite colonizes the intestine and causes diarrhea and malabsorption are not well understood (5). However, we have recently shown that ox bile and pig bile accelerate growth of *Giardia* in vitro (6), which suggests that bile may be an important colonization factor for this parasite. The purpose of the present study was to determine in vitro the mechanism by which mammalian bile stimulates growth of *Giardia* trophozoites.

We have approached this problem in a number of complementary ways. First, we have extended our previous observations on native bile by studying growth of *Giardia* in the presence of human and guinea pig bile, the former containing mainly glycine conjugated bile salts and the latter being essentially devoid of phospholipid and relatively deficient in common bile salts (7, 8). Second, we have investigated individually and together the effects of the major biliary lipids (bile salts, lecithin, and cholesterol) on growth of *Giardia;* and third, we have measured membrane lipid uptake by the parasite in the presence and absence of native bile and pure bile salts. Finally, we have studied uptake of conjugated bile salts themselves by *Giardia* trophozoites.

Methods

Materials

Giardia lamblia (axenic Portland 1 strain) was kindly provided by Dr. L. Diamond, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

Culture medium. Giardia trophozoites were adapted to grow in a modified culture medium (TYI-S; Trypticase, yeast extract, iron-serum) originally devised for cultivation of *Entamoeba histolytica* (9). Its major constituents are a pancreatic enzyme digest of casein and yeast extract (Biosate Peptone, Baltimore Biological Laboratories, Cockeysville, MD), heat-inactivated bovine serum (final concentration, 10%, vol/vol) (Biofluids, Rockville, MD), and D-glucose, ferric ammonium citrate, L-cysteine, ascorbic acid, and sodium chloride (Sigma Chemical Co., St. Louis, MO). The modifications of this medium used in the present study were (*a*) Diamond's TPS-1 vitamin solution (North American Biologicals, Miami, FL) replaced the vitamin-Tween 80 solution, (*b*) the culture medium was not autoclaved but sterilized by passage through a 0.22-μm nitrocellulose filter (Millipore Corp., Bedford, MA) and (*c*) antibiotics, because of surface activity, were omitted.

Bile and biliary lipids. Immediately after animals were slaughtered in local abattoirs, ox and pig gallbladder bile was collected into sterile glass vessels and kept on ice. Guinea pigs (Duncan Hartley strain, 450 g) were killed by a blow on the head and gallbladder bile was obtained by hypodermic aspiration into a sterile syringe. Human common hepatic duct bile was obtained from one individual undergoing therapeutic transhepatic biliary drainage. Biles were centrifuged at 1,000 *g* for 30

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min and sterilized by passage through 0.2 μm nitrocellulose membrane (Synbron/Nalge, Rochester, NY). Total solids in bile were determined by dessication and weighing of a known volume. Bile was freeze-dried and stored at 4°C. Freeze-dried ox bile was also obtained from Sigma Chemical Co. Dried bile was reconstituted as required with sterile, deionized water.

Egg yolk phosphatidylcholine (lecithin) (Grade 1; Lipid Products, Surrey, England) shown previously to be >99% pure (10) was stored in the dark in chloroform-methanol (2:1 vol/vol) in sealed glass vials at −20°C under nitrogen. Soybean lecithin was purchased from Sigma Chemical Co., and sodium glycocholate (GC), sodium taurocholate (TC), and sodium taurodeoxycholate (TDC) were purchased from Calbiochem-Behring Corp., La Jolla, CA. Minor unconjugated bile acid impurities found previously in this TDC preparation were removed by standard methods (10), while GC and TC were used in the same form as they were received. L-α-1-palmitoyl-2-oleoyl-[oleoyl-1-^{14}C]lecithin (specific activity [SA], 40–60 mCi/mmol; 98.5% pure by thin-layer chromatography [TLC]), [1,2,6,7-^{3}H (*N*)]cholesterol (SA, 60–90 Ci/mmol; 94% pure by TLC and high pressure liquid chromatography [HPLC]), [^{3}H (G)]taurocholate (SA, 2–5 Ci/mmol; 98% pure by TLC), and Aquasol were purchased from New England Nuclear, Boston, MA. Radiochemicals were used as received.

Procedures

Cultivation, harvesting, and determination of generation time of Giardia trophozoites. Giardia were cultivated and harvested as we have described previously (6, 11). In experiments in the present study, new cultures were initiated by addition of 1.5–2.5 × 10^4 trophozoites per tube. Growth of *Giardia* in culture was assessed by serial determinations of trophozoite concentration (counted in a Spencer Bright-line hemocytometer) such that generation or "doubling" time (g) of the organism could be determined (11). Growth of *Giardia* was assessed during a 48-h period after supplementation of TYI-S medium with mammalian bile (ox, pig, guinea pig, and human, 5 mg–4 g/liter), pure bile salts (0.2 μm–2.0 mM), detergents Triton X-100 and Tween 80 (0.0002–0.06%), and soybean and egg lecithin (0.01–1.0 mM). Lecithins were dispersed in the culture medium without sonication and therefore were present as multilamellar liquid crystalline vesicles. The combined effect of GC (2 mM) and soybean lecithin (0.1–0.5 mM) on parasite growth was also examined.

Uptake of lecithin, cholesterol, and taurocholate by Giardia. The uptakes of lecithin and cholesterol were determined separately using tracer quantities (~10^5 dpm) or ^{14}C-labeled palmitoyl-oleoyl lecithin and ^{3}H-labeled cholesterol added to the lipid containing TYI-S culture medium, and after supplementation of this medium with commercial ox bile (0.8 g/liter), egg lecithin (0.05 mM), and pure TC (0.2 mM). Membrane lipid uptake was also determined after further ('booster') administration of TC (0.2 mM) to the TC supplemented cultures at 24 and 48 h. After each growth period trophozoite numbers per tube were determined, parasites were sedimented by centrifugation (500 *g*, 15 min), and were washed three times in 0.01 M phosphate-buffered saline (PBS). The final pellet was solubilized in 200 μl of 0.1 M KOH and was prepared for liquid scintillation spectrometry by addition of 4.0 ml Aquasol. In addition to zero-time controls, control tubes without parasites were similarly treated to determine nonspecific binding of radiolabel to the tube walls.

Trophozoite uptake of TC was determined during 48-h culture, using tracer amounts of ^{3}H-labeled TC added to cold TC provided by either ox bile (final TC concentration, 0.38 mM) or pure TC (0.2 mM). Methods were otherwise identical to those described for other membrane lipids.

Growth stimulation and lecithin uptake by Giardia. Commercial ox bile (0.025–0.8 g/liter) was added to native TYI-S medium and *Giardia* trophozoites were cultivated for 48 h. For each concentration of bile, generation time was calculated and lecithin uptake was determined by addition of ^{14}C-lecithin tracer (~10^5 dpm) to the culture medium.

Lipid analyses. Culture media and biles (ox, pig, and guinea pig) were analyzed for bile salt, phospholipid, and cholesterol composition. Individual and total bile salts were quantified by reverse-phase high performance liquid chromatography (12), phospholipids were measured directly as inorganic phosphorus by the method of Bartlett (13) with subtraction of buffer phosphate and analyzed by Wuthier's two dimensional silica gel-impregnated paper chromatography (14). Choline-containing phospholipids were also analyzed by the choline oxidase method (15). Cholesterol was determined by Carr and Dreckter's modification (16) of the original method of Abell et al. (17).

Statistics. Results are expressed as the mean±1 SEM. Unpaired *t* tests were used to evaluate differences.

Results

Giardia growth experiments

Effect of native mammalian bile. All biles (ox, pig, guinea pig, and human) stimulated growth of *Giardia* in the presence of TYI-S culture medium as exemplified by a substantial reduction in generation time during a 48-h culture (Fig. 1). However, this effect was strongly concentration-dependent, with maximal stimulation (lowest generation times) occurring at concentrations of 0.1–0.8 g/liter (Fig. 1). Commercial ox bile (data not shown) had similar effects, although maximal growth stimulation occurred at 0.8 g/liter. At higher concentrations of bile (>0.8 g/liter), generation time increased (growth retardation). This effect was most marked with ox bile. When optimal stimulatory concentrations of biles (guinea pig, 0.8 g/liter; other biles, 0.1 g/liter) were compared (n = 6–36 experiments), guinea pig bile was significantly less effective in reducing mean generation time of bile-free control medium (8.9±0.3 h vs. 11.7±0.6 h; $P < 0.05$) than human (7.9±0.1 h; $P < 0.05$), pig (8.1±0.1 h; $P < 0.05$), and ox (7.6±0.2 h; $P < 0.01$) biles.

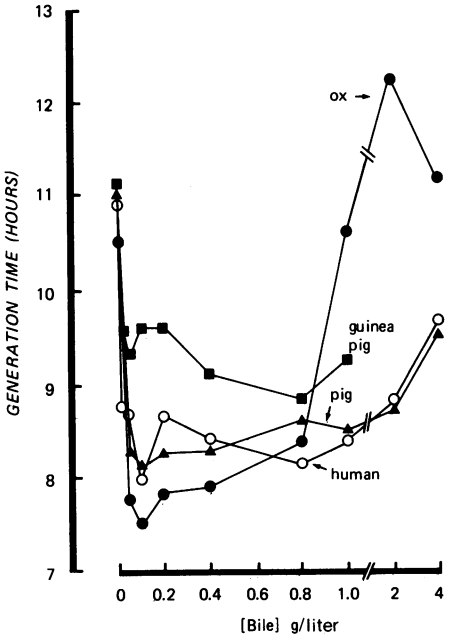

Figure 1. Influence of four mammalian biles on in vitro growth of *Giardia*. *Giardia* growth acceleration and inhibition by supplementation of native TYI-S culture medium with fresh ox, pig, guinea pig, and human bile (5.0 mg–4.0 g/liter). Lowest generation times (i.e., greatest acceleration of growth) for ox, pig, and human bile were achieved at 0.1 g/liter, and those for guinea pig bile were achieved at 0.8 g/liter. Ox bile inhibited growth at concentrations > 2 g/liter.

1. *Abbreviations used in this paper:* CMC, critical micellar concentration; GC, glycholate; SA, specific activity; TC, taurocholate; TDC, taurodeoxycholate; TLC, thin-layer chromatography.

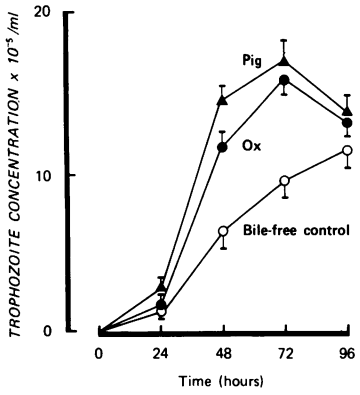

Figure 2. Influence of time (h) on growth of *Giardia* expressed as trophozoite concentration per unit time. Complete *Giardia* growth curves for native TYI-S medium and after supplementation with fresh pig bile or ox bile at optimal growth promoting concentration (0.1 g/liter).

Fig. 2 shows the time dependence of *Giardia* trophozoite growth expressed as parasite concentrations at 24-h intervals for 96 h in native culture medium and with optimal concentrations of fresh ox bile and pig bile. After 48 h and 72 h trophozoite concentration was 2.0–2.5-fold greater with bile-supplemented media than control. A semi-log plot (not displayed) showed that after innoculation of culture medium with 1.5–2.5 × 10^4 organisms, log-phase growth persisted for 48 h. All subsequent growth experiments with and without mammalian biles and their pure lipid components were examined during log-phase growth; generation times were calculated between 0 and 48 h.

Our biliary lipid analyses confirmed published data (7, 8) that there are quantitative and qualitative differences between guinea pig and other mammalian biles (Table I). Guinea pig bile contains little or no phospholipid, minimal amounts of cholesterol, and at least 50% of its bile salts are 7-keto-lithocholates, which are poor detergents and therefore are less effective solubilizers of lecithin. Cholesterol and lysolecithin concentrations in TYI-S medium were similar for all biles, indicating that differences in growth promotion cannot be attributed to these lipids (Table II).

Effect of pure bile salts. As depicted in Fig. 3, addition of pure conjugated bile salts (TC, GC, and TDC) to the lecithin/cholesterol containing culture medium influenced generation time and growth of *Giardia.* The more hydrophilic trihydroxy bile salts, TC and GC, had little effect on growth at low concentrations but stimulated growth as the concentration approached the critical micellar concentration (CMC; ~3.0 mM) (18). The more hydrophobic dihydroxy bile salt, TDC, accelerated growth at even the lowest concentrations examined; as the concentration approached the CMC (~0.8 mM) (18), growth was inhibited. The growth acceleration with TC and GC was 10–15% less than that obtained with native ox bile. Growth was not promoted by low concentrations of Triton X-100 or Tween 80 and was markedly inhibited at concentrations >0.02%.

Effect of lecithin supplementation. Phospholipid concentration in the native TYI-S culture medium was 0.027 mM (Table II). Supplementation with egg and soybean lecithins to give total final lecithin concentrations up to 0.05 mM (approximate lecithin concentration obtained after supplementation with ox bile, 0.1 g/liter) was without effect, although 0.1–0.4 mM lecithin modestly accelerated growth (6.1–8.6%). However, addition of GC (2 mM) to the lecithin-supplemented medium produced stimulatory effects that were not different from GC alone.

Lipid uptake experiments

Effect of native bile. Fig. 4 displays that during 72-h culture periods, uptake of lecithin by *Giardia* trophozoites was significantly greater with commercial ox bile-supplemented (0.8 g/liter) TYI-S medium than from bile-free control medium. The effect was most marked during the first 24 h. Uptake of cholesterol was similar from control and ox bile-supplemented media, although at 24 h cholesterol uptake from the bile-enriched medium was significantly lower than from control medium.

Effect of taurocholate. Fig. 5 shows that addition of a single, initial submicellar concentration of TC (0.2 mM) did not significantly increase lecithin uptake by *Giardia* trophozoites. Lecithin uptake was significantly enhanced at 72 h after additional TC (0.2 mM) had been added at 24 and 48 h. Cholesterol uptake was similar in control, TC supplemented medium, and TC 'boosted' medium (Fig. 5). Because "boosting" the culture media with TC enhanced lecithin uptake by *Giardia,* we tested the possibility that TC was itself consumed during growth of the organism. TC added as pure bile salt was taken up by *Giardia* trophozoites throughout the 48 h incubation (not displayed). This effect was most marked at 4 h, when 138.3±6.8 nmol of TC were consumed per 10^8 trophozoites (mean±SEM, n = 3 experiments). Uptake of TC was similar at 24 and 48 h and was substantially greater when added at 0.2 mM as pure bile salt (129.3±44.3 nmol/10^8 trophozoites in 24 h) than when added at 0.38 mM in dilute bovine bile (5.3±2.6 nmol/10^8 trophozoites in 24 h), which suggests that the physical state of bile salts is important in determining uptake.

Growth stimulation and lecithin uptake. Fig. 6 shows the relationship between enhanced lecithin (from added ox bile) uptake and reduction in *Giardia* generation time and thus acceleration of growth. Lecithin uptake was greatest from culture medium containing highest concentrations of lecithin and bile salts. This curve has the form of a rectangular hyperbole, which suggests that there was a substantial reduction in generation time before a major increment in lecithin uptake was detected.

Table I. Lipid Composition of Native Mammalian Bile

Bile	Dry weight	Total bile salts	Cholesterol	Phosphatidyl Choline	Phosphatidyl Serine	Phosphatidyl Inositol	Lysolecithin
	g/liter	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
Ox*	68.0	94.6	2.30	24.0	0.04	0.01	1.66
Pig*	94.0	156.0	3.21	18.1	0.74	0.08	0.18
Guinea pig*	24.0	17.9	0.28	0.14	<0.01	<0.01	<0.01
Human‡	11.3	27.8§	4.43§	20–7.25§			0.01–0.15§

* Gallbladder bile. ‡ Common duct bile. § Data from reference 10. All data refer to fresh (not commercial) bile.

Table II. Final Composition of Native and Bile-supplemented TYI-S Culture Medium*

Bile supplement	Total bile salts	Cholesterol‡	Phosphatidyl choline‡	Lysolecithin
g/liter	*mM*	*mM*	*mM*	*mM*
Ox (0.1)	0.14	0.203	0.062	0.012
Pig (0.1)	0.17	0.203	0.046	0.010
Guinea pig (0.8)	0.60	0.209	0.031	0.010
Human§ (0.1)	0.25	0.239	0.091	0.011
Native TYI-S	ND	0.200	0.027	0.010

* Bile concentration added (see first column) was that which optimally stimulated growth of *Giardia* in vitro (see Figs. 1 and 3).
‡ Concentration much higher than in native bile due to high background lipid concentrations in TYI-S culture medium.
§ Human common duct bile was not analyzed but approximate composition was computed from dry weight (Table I) and data from reference 10.
ND, not detected. Theoretical total bile salt concentration in TYS-I medium containing 10% serum would be <100 nM, and for practical purposes this was not included in calculations relating to bile salt content of supplemented media.

Addition of pure egg lecithin (final lecithin concentration, 0.05 mM) alone to TYI-S medium also substantially increased lecithin uptake at 48 h (187.5±0.13 nmol/10^8 trophozoites compared with control, 29.9±0.04 nmol/10^8 trophozoites, n = 3), but generation time was similar at 12.0±0.3 h and 12.0±0.2 h, respectively. Lecithin uptake from egg lecithin-supplemented medium was also greater than from ox bile (77.5±0.11 nmol/10^8 trophozoites).

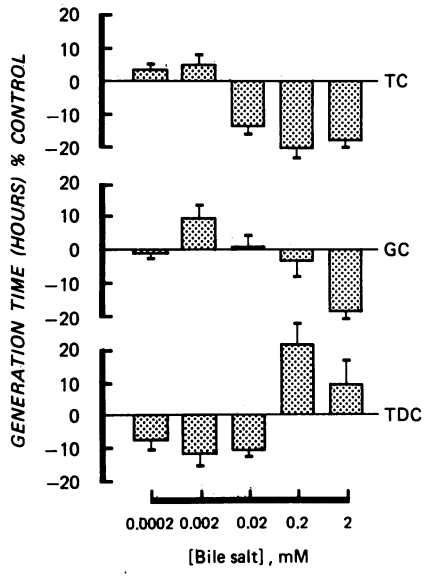

Figure 3. Influence of pure bile salts on generation time (h) of *Giardia* grown in TYI-S medium. Stimulation of growth by TC, GC, and TDC (mean±SEM, n = 3 experiments) is expressed as a percentage of control generation time obtained with TYI-S medium alone. Thus, growth acceleration is denoted by negative values and inhibition by positive values. TC and GC reduced generation time as bile salt concentration approached the CMC (~3.0 mM), whereas TDC stimulated growth at concentrations below the CMC (~0.8 mM) but retarded growth at higher concentrations. Maximal reduction in generation time with pure bile salts (~20%) was less than that observed with native ox bile (30–35%).

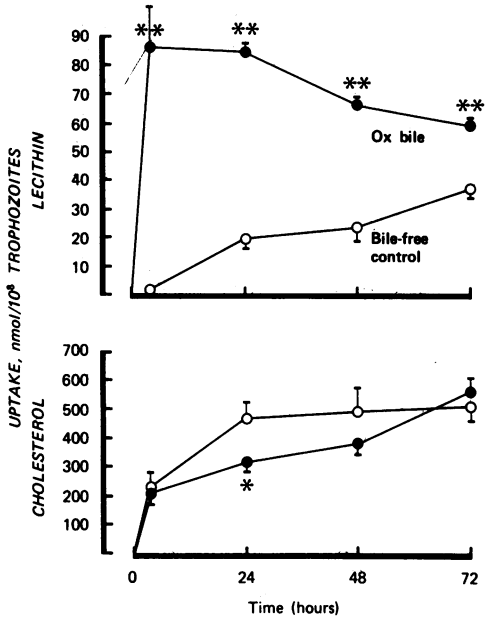

Figure 4. Time course of uptakes of lecithin and cholesterol by *Giardia* trophozoites from bile-free control TYI-S medium and after supplementation of this medium with commercial ox bile (0.8 g/liter). Lecithin concentration in native TYI-S medium was 0.027 mM and was 0.22 mM in the bile-supplemented medium. Cholesterol concentration (0.2 mM) was the same in both media. Each determination represents the mean±SEM of n = 3 experiments, each performed in triplicate. *, $P < 0.05$; **, $P < 0.02$.

Consumption of lipid from culture medium

During 72-h growth studies (n = 3), total phospholipid concentration in the culture medium decreased linearly (0.16±0.01 to

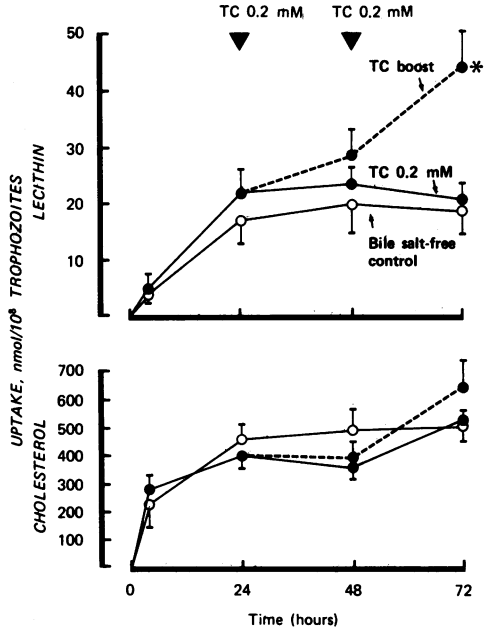

Figure 5. Uptake of lecithin and cholesterol by *Giardia* trophozoites from native and TC-supplemented (0.2 mM) TYI-S medium. TC was added to all TC tubes at time zero, but further supplements ("boosting") of TC were made at 24 h and 48 h (broken line). Each uptake determination represents the mean±SEM of n = 3 experiments, each performed in triplicate. *, $P < 0.05$.

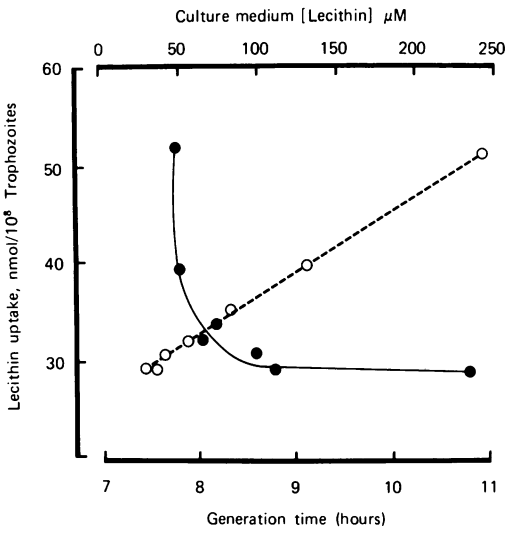

Figure 6. Relationship between lecithin uptake by *Giardia* trophozoites and growth stimulation (●) as judged by reduction in generation time (n = 3). *Giardia* was grown for 48 h in commercial ox bile (25–500 mg/liter) at which time lecithin uptake was determined. Generation time apparently decreased before marked differences in lecithin uptake were observed. Lecithin uptake by trophozoites was linearly related to lecithin concentration in the culture medium (○), which supports the view that the lecithin concentration of the medium is not rate-limiting for growth and enhanced lecithin uptake is not the primary growth promoter.

0.09±0.02 mM), while cholesterol concentration was unchanged. This suggests that there was net phospholipid, but not cholesterol uptake during growth and multiplication of *Giardia* trophozoites.

Discussion

The recent observation that *Giardia* is unable to synthesize membrane phospholipid (4) suggested to us that biliary phospholipid in the duodenum and proximal jejunum might be an important source of preformed membrane phospholipid for the parasite, and thus, a key factor governing its choice of habitat in the proximal small intestine. Indeed, earlier biliary diversion studies in the rat in vivo had strongly suggested the dependency of *Giardia* growth on luminal bile (19). In preliminary studies we and others have shown that ox and pig bile stimulated in vitro growth of *Giardia* (6, 20), and it seemed that the growth factors were probably the major biliary lipids (bile salts, lecithin, and cholesterol).

The aim of the present study, therefore, was to systematically examine the effect of each biliary lipid as well as native bile on parasite growth. We measured *Giardia* growth in the presence of mammalian biles with different relative lipid compositions and "spectrum" biliary lipids. Guinea pig bile, which is virtually devoid of phospholipid (7, 8), stimulated growth but was significantly less effective than ox bile. Thus, although biliary phospholipid may contribute to the growth promoting effects of bile, other components such as bile salts are also likely to be important, although the individual effect of lecithin could not be exclusively examined due to the background phospholipid content of the culture medium. Guinea pig bile, in addition to the absence of phospholipid, contains a high proportion of 7-keto-lithocholic acid, which has a CMC in the 18–25 mM range (21) and a paucity of common more hydrophobic bile salts (7, 8). To clarify these issues, we studied the individual growth effects of some of the major constituents of mammalian bile on growth of *Giardia*.

Common bile salts (GC, TC, and TDC) stimulated growth of *Giardia* in these in vitro studies. This observation is consistent with the finding more than 30 years ago that feeding GC to rats with natural *Giardia muris* infections increased parasite numbers in the small intestine (22). Bile salts, however, failed to reproduce completely the growth stimulatory effects of native bile, suggesting that other components of bile may be contributary. Addition of lecithin to the lecithin containing culture medium (0.027 mM) failed to accelerate growth when added at the same concentration as found in optimal stimulatory concentrations of ox bile. When combined with an optimal concentration of GC, the growth promoting effects were no better than with GC alone. Thus, in the presence of bile salts, the lecithin content of the culture medium is not rate-limiting for growth. Increasing lecithin concentration in the medium, however, in the absence of bile salts did seem to facilitate growth, presumably by increasing the lecithin concentration gradient between medium and parasite. Supplementation experiments with cholesterol were not performed, because the cholesterol content of the medium was high (0.2 mM) and adding dilute bile failed to make a substantial contribution to the final cholesterol concentration (Table II).

If bile salts were the major, primary growth stimulators, as the above experiments indicate, it seemed likely that they were having their effect by facilitating transfer of membrane lipid into *Giardia* membrane. Lipid uptake experiments confirmed that lecithin but not cholesterol uptake was promoted in the presence of both native bile and pure TC, although in pure TC experiments cultures required interval "boosting" with TC to demonstrate this effect (Fig. 6). The need to "boost" cultures with TC before observing enhanced lecithin uptake may be explained by the parasite's ability to consume TC. Further studies have shown that *Giardia* also consumes GC, and TLC analysis confirms that these bile salts are unchanged after parasite uptake (23).

Our inability to totally reproduce with pure salts the stimulatory capacity of native bile may be related to the formation of large unilamellar vesicles (of mixed lipids saturated with bile salts) that would exist when bile was highly diluted in the native bile-supplemented culture medium (24). Presumably this would provide a more physiological and therefore more effective vehicle for the promotion of membrane lipid insertion into *Giardia* membrane. Biliary lipids would be in a similar physical state in the proximal small intestine (24).

Although these findings are consistent with our hypothesis that bile salts enhance membrane lipid uptake, notably lecithin uptake, and therefore stimulate parasite growth, there are several problems that must be considered. First, the use of radiolabeled tracers to monitor lipid movement between medium and parasite does not distinguish between mass transfer and exchange. Our findings suggest exchange only in the case of cholesterol but true net uptake in the case of lecithin. We did not detect lecithin degradation during these experiments (data not shown) and would not expect it, since we avoided using powerful sonication in the preparing experimental culture media, and phospholipase A_2 activity was absent.

A second question relates to the relationship between lecithin uptake and the rate of multiplication of *Giardia*. *Giardia* growth was stimulated by low concentrations of ox bile at which increased lecithin uptake could not be demonstrated; thus, reduction in generation time preceded a substantial increment in lecithin uptake. The explanation for this finding is not clear although

we cannot exclude the possibility that low concentrations of bile salts have a primary growth stimulating effect that is independent of their apparent facilitatory action on lecithin uptake. This view is supported by our finding that other detergents failed to stimulate growth of *Giardia*. Our observation that lecithin supplementation of culture medium (up to 0.05 mM) in the absence of bile salts increased lecithin uptake (probably by nonspecific adsorption) without enhancing parasite growth also supports a major primary role for bile salts in growth promotion. Ideally we would have examined the growth effects of bile salts in a lecithin-free culture medium but at present this is not possible, as *Giardia* only multiplies in the presence of serum, and replacing serum with lecithin does not result in a medium that supports growth (Farthing, M. J. G., unpublished observations).

These results contribute to our understanding of some well established clinical observations relating to *Giardia* infection in humans and animals. The abundance of dilute bile in the duodenum and proximal jejunum (24), we presume, is biologically advantageous to the parasite and may explain the favored geographic location of this parasite in the upper small intestine (2, 3). However, we cannot exclude the possibility that other intraluminal substrates in the proximal small intestine may also be trophic for *Giardia* and thus may influence its colonization site. Although impaired intestinal absorption of dietary fat is well documented in giardiasis (25, 26), the mechanisms involved are not fully understood. There is evidence that intestinal mucosal damage (27, 28) and concomitant bacterial overgrowth with bile salt deconjugation (29, 30) are contributory factors. *Giardia* does not itself deconjugate bile salts (23, 31), but according to our observations it may reduce intraluminal bile salt concentrations by direct uptake, and thus contribute to fat malabsorption.

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