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Attachment of the Flagellate *Giardia lamblia*: Role of Reducing Agents, Serum, Temperature, and Ionic Composition

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The flagellated protozoan *Giardia lamblia* has been grown only in highly complex media under reduced oxygen tension. Therefore, the organic and physiological requirements for in vitro attachment and short-term (12-h) survival of this organism were determined. In defined maintenance media, a thiol reducing agent (e.g., cysteine) was absolutely required for attachment and survival of this aerotolerant anaerobe. The crude bovine serum Cohn III fraction greatly stimulated attachment and survival. Attachment was decreased at a reduced temperature (24°C as compared with 35.5°C) and absent at 12°C or below. Attachment and survival were strongly dependent upon pH and ionic strength, with optima at pH 6.85 to 7.0 and 200 to 300 mosmol/kg. Sodium chloride was better tolerated than KCl. Reduction of Ca^{2+} and Mg^{2+} to below 10^{-8} M did not significantly affect attachment.

Giardia lamblia is an actively motile, flagellated parasitic protozoan which attaches to the human intestinal epithelium during infection (15). *Giardia* trophozoites attach to surfaces via the ventral adhesive or striated disk, which contains contractile proteins (5, 12; S. L. Erlandsen, J. V. Schollmeyer, D. E. Feely, and D. G. Chase, J. Cell Biol. **79**:264a, 1978; D. E. Feely and S. L. Erlandsen, J. Cell Biol. **87**:218a, 1980). In vitro, *Giardia* trophozoites also attach rapidly and tenaciously to glass or plastic (4, 10, 18). During logarithmic growth in culture, >90% of the trophozoites may attach, eventually forming a confluent monolayer (10, 18). Attached organisms are active; some of their four pairs of flagella continue to beat (12). They may also be observed to detach and reattach spontaneously. In addition, chilling quantitatively releases attached *Giardia* trophozoites, which is the basis of a convenient attachment assay (4, 10; Feely and Erlandsen, J. Cell Biol., **87**:218a, 1980).

Although attachment is clearly important for *Giardia* trophozoites, the physiological requirements for this active process are not understood. A major obstacle has been that, to date, this aerotolerant anaerobe has been grown only in highly complex media (14, 18) with thiol reducing agents required for growth, attachment, and resistance to oxygen (9, 10). Simpler media which support short-term (~12-h) survival and apparently normal attachment and motility have not been available.

The present study demonstrates that, in several defined media, trophozoite survival and attachment were dependent upon thiol reducing agents and highly stimulated by a serum fraction. The effects of temperature, pH, and ionic composition were also determined.

MATERIALS AND METHODS

Organisms and culture. *G. lamblia* (Portland 1, ATCC 30888), isolated from the duodenal fluid of a patient with diarrhea (14), was obtained from Visvesvara and Healy (Centers for Disease Control, Atlanta, Ga.) and grown in modified (10, 18) Diamond TP-S-1 medium (2) with bovine serum (3).

To maintain low oxygen tensions, screw-capped culture tubes (16 by 125 mm; disposable borosilicate glass) nearly filled with medium (13 to 15 ml) were used. Subcultures were made twice weekly. Trophozoites were grown to log phase at 35.5 ± 0.5°C for 24 to 72 h (depending upon the inoculum) for all experiments. Cultures were determined to be in logarithmic phase by microscopic examination, determination of cell concentration, and reference to standard growth curves.

Attachment studies. A non-growth maintenance medium (maintenance medium 2 [MM-2]) whose components could be varied easily was used in all studies (Table 1). The media were prepared for each experiment from concentrated salt, protein, and vitamin solutions. Reducing agents were freshly prepared for each experiment. All labile components were filter sterilized. In each experiment, the component to be varied was omitted from the medium used to wash, suspend, and distribute the cells, designated incomplete maintenance medium (IMM).

Only attached cells were used in the experiments;

TABLE 1. Components of MM-2

Component[a]	Concn
Tris-hydrochloride, pH 7.0	50 mM
$MgCl_2$	8 mM
$CaCl_2$	2 mM
NaCl	25 mM
KCl	12 mM
Vitamin solution no. 107	2.5% (vol/vol)
L-Cysteine[b]	6 mM
L-Ascorbic acid[b]	6 mM
Cohn fraction III	1 mg/ml

[a] The first five components were diluted from a 5× concentrated stock mixture.

[b] Freshly prepared and neutralized.

these cells were obtained in the following manner. Culture tubes were inverted twice, and the growth medium and free organisms were discarded. IMM was added to the tubes. The adherent organisms were made to detach by chilling the cultures for 10 min in an ice water bath and inverting 10 times. The cells were washed by centrifugation (10 min, 10°C, 2,400 × *g*) and suspended in IMM. Samples of cell suspensions were enumerated with a model Z_{B1} electronic counter (Coulter Electronics, Inc., Hialeah, Fla.). To initiate experiments, concentrated trophozoite suspensions (0.4 ml) in IMM were added to 1-dram (4.7-ml) borosilicate glass screw-capped vials containing medium supplemented with the appropriate experimental components (3.6 ml). The exclusion of most of the air was important for survival (10). After mixing, the cultures were incubated upright at 35.5 ± 0.5°C, and the numbers of viable and attached organisms were determined in parallel cultures as described below.

(i) Attachment assay. The attachment of individual *G. lamblia* trophozoites, as observed microscopically, may take only a few seconds (5; Erlandsen et al., J. Cell Biol. **79**:264a, 1978; Feely and Erlandsen, J. Cell Biol., **87**:218a, 1980). The number of attached organisms in a population at a given time is a function of the relative rates of attachment and detachment. A goal of the studies reported here was to identify those conditions under which the maximum number of parasites attach. Since preliminary studies showed that, in MM-2, the maximum number of cells was attached after 2 h of incubation, this time was used for attachment assays, unless otherwise specified. Cultures for determination of attachment and survival were set up in parallel. The attachment cultures contained 5×10^4 to 1×10^5 trophozoites per ml, which yielded reliable numbers of cells for electronic counting. After incubation at 35.5°C, the medium and nonattached trophozoites were removed by vacuum aspiration. The vials were then rinsed with 1 ml of warm medium which did not remove firmly attached trophozoites. The trophozoites were made to detach in 1 ml of cold phosphate-buffered (pH 7.2) 0.85% saline by chilling for 10 min and shaking. The entire sample was diluted in counting buffer and counted electronically as described above (10). This assay does not distinguish between viable and dead trophozoites.

(ii) Determination of viability. Assays of survival which are based on trophozoite motility or dye exclusion or both are not suitable for these studies for the following reasons. First, some trophozoites attach, whereas others move too rapidly to be counted accurately. Second, motile *G. lamblia* trophozoites may not be alive by the more stringent definitions of intactness or ability to multiply. We frequently observed aberrantly shaped trophozoites (sickled or balloon-like) which were motile but unable to multiply. The extreme case is the preparation of motile cytoskeletons from Triton-extracted *Giardia* trophozoites (12). Finally, microscopic assays are too time consuming; viability may decrease during the determination.

The ability of a single cell to multiply and form a colony is a stringent criterion for viability. The recently developed colony assay for survival of *G. lamblia* trophozoites was used (7, 10). After incubation for suitable times (1 to 24 h) according to the protocol, vials containing 5,000 trophozoites per ml were chilled in ice water for 10 min to release attached parasites and mixed to create a single cell suspension. Duplicate cell samples were transferred to molten agarose growth medium as described (10). Each vial was used only once. After the medium solidified, viable single cells suspended in the semisolid matrix grew into visible colonies in 5 to 7 days (7). The colony-forming efficiency (the number of colonies divided by the number of cells inoculated) was approximately 50%. Except where otherwise specified, viability was assayed after 10 h of incubation. This time was chosen because one of the goals of these studies was to maximize and prolong parasite survival. Frequently, the effects of nonoptimal conditions (e.g., pH and ionic strength) on survival were not apparent at 2 h, when attachment was usually assayed, but became evident after longer incubation. Thus, differences in attachment were maximal at 1 to 2 h, a time when viability remained high (except in the absence of reducing agents). The greatest differences in viability were apparent at approximately 10 h.

Quantitation. It must be recognized that the precision of the attachment and survival data generated in a given experiment may exceed the reproducibility of the results from experiment to experiment. This is especially true in physiological experiments involving fastidious organisms grown in complex media. Within each experiment, however, there was little variation between duplicates (usually less than ±10% of the value). Therefore, absolute results may be compared only within a given experiment. The averages of duplicate determinations were used, and differences of less than 20% were not considered to be significant. The variables which were considered to be important were those which produced at least twofold increases in attachment or survival or both. All results shown were typical of two to five experiments.

Reagents. Eagle minimal essential medium was purchased from Flow Laboratories, Inc., Rockville, Md.; RPMI 1640 medium and medium 199 (without $NaHCO_3$ or serum, with 25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid] and Hanks salts) were obtained from GIBCO Laboratories, Grand Island, N.Y.; and bovine serum was obtained from Biofluids, Inc., Rockville, Md. The serum was inactivated at 56°C for 50 min. The vitamin mixture of medium NCTC 107 (2) was prepared by the National Institutes of Health Media Unit. Gastric

mucin (porcine) was obtained from Wilson Laboratories, Chicago, Ill.; bovine serum albumin was obtained from Armour Pharmaceutical Co., Kankakee, Ill.; transferrin was obtained from Miles Laboratories, Inc., Elkhart, Ind.; and beta-lipoprotein and fibrinogen were obtained from ICN Nutritional Biochemicals, Cleveland, Ohio. Cytochalasin B, C, and D (Aldrich Chemical Co., Milwaukee, Wis.) were dissolved in dimethyl sulfoxide and diluted into MM-2. Human fibronectin (lot no. 08080; Bethesda Research Laboratories, Bethesda, Md.) was dissolved in 0.1 M 3-(cyclohexylamino)propanesulfonic acid, pH 11, and then diluted into MM-2 with 20 mM HEPES, pH 7.2, to control the final pH.

Cohn bovine serum fraction III (Cohn III; lot no. 26C 0197-1) and other reagents were purchased from Sigma Chemical Co., St. Louis, Mo. Cohn III purchased from a source other than Sigma was not active in supporting attachment and survival of *G. lamblia* trophozoites.

Osmolality was determined with a model 3R osmometer (Advanced Instruments, Inc., Needham Heights, Mass.), and pH was determined with a Corning pH meter 130.

Separation of Cohn III high- and low-molecular-weight fractions was done with an immersible CX ultrafiltration unit with a 10,000-molecular-weight pore size (Millipore Corp., Bedford, Mass.).

RESULTS

The effect of physiological factors such as pH and osmolality and biochemical factors such as reducing agents, vitamins, and serum fractions upon adherence of *G. lamblia* trophozoites to glass and survival were determined by varying the components of MM-2 (Table 1). The effects of some of the biochemical factors were also tested in several tissue culture media.

Reducing agents. The ability of *G. lamblia* trophozoites to attach to glass in the absence of growth and to survive was absolutely dependent on thiol reducing agents (Table 2). Support of attachment and survival by D-cysteine, thiomalic acid, α-mercaptopropionyl glycine, glutathione, and *N*-acetyl cysteine did not differ greatly from that by L-cysteine. Thus, the requirement for a thiol was not highly specific. In contrast, ascorbic acid was completely ineffective alone, although in the presence of a thiol reducing agent, it stimulated both attachment and survival. The thiol-blocked analog *S*-aminoethyl cysteine, as well as cysteine methyl ester, which has a free thiol, was ineffective. Cysteine methyl ester was toxic to *G. lamblia* trophozoites in complete growth medium (9).

Serum factors. Bovine serum was highly stimulatory to attachment and survival of *G. lamblia* trophozoites in several tissue culture media as well as in the simpler MM-2 (Table 3). A defined vitamin solution was stimulatory of both attachment and survival in the absence of serum in MM-2. In the presence of serum, however, the vitamin mixture did not provide additional stimulation (data not shown).

It was of interest to determine which components or fractions of serum stimulated parasite attachment. Of the bovine serum fractions tested in MM-2, the crude Cohn III was most stimulatory of attachment and was the only fraction to support survival (Table 4). The stimu-

TABLE 2. Effects of reducing agents on attachment and survival of *G. lamblia* trophozoites in MM-2

Addition[a]	No. of cells attached × 10^3[b]		No. of survivors: colonies per ml[c]	
	Without ascorbic acid	With ascorbic acid[d]	Without ascorbic acid	With ascorbic acid[d]
None	3.7	3.2	0	0
Ascorbic acid	5.3	11.4	0	0
L-Cysteine[e]	51.2	89.6	1,533	1,820
D-Cysteine[e]	55.2	65.9	1,440	2,160
α-Mercaptopropionylglycine[e]	45.5	75.0	760	2,120
Thiomalic acid[e]	50.0	69.8	1,393	1,840
Glutathione[e]	52.7	66.9	1,267	1,760
N-Acetyl cysteine[e]	41.0	56.9	813	1,620
Thioglycolic acid[e]	49.5	28.8	420	360
Cysteine methyl ester	4.3	4.8	0	0
S-Aminoethyl cysteine	6.3	2.7	0	0
Methionine	6.8	15.2	0	0
Growth medium[f]	—[g]	82.9	—	3,133

[a] All components were added at a concentration of 6 mM, except ascorbic acid, which was added at a concentration of 50 mM.

[b] Incubation was for 3 h in MM-2 prepared without reducing agents and supplemented as indicated.

[c] Incubation was for 10 h.

[d] Ascorbic acid at a concentration of 10 mM.

[e] These thiol reducing agents were considered active.

[f] Complete TP-S-1 medium with serum.

[g] —, This medium contains 1.2 mM ascorbic acid.

TABLE 3. Effects of serum on attachment and survival of *G. lamblia* trophozoites in various media

Medium[a] and addition	No. of cells attached × 10^3 at 2 h	No. of survivors: colonies per ml at 11 h[b]
NCTC 135		
None	37	0
Serum[c]	53	3,340
NCTC 199		
None	21	730
Serum	74	2,420
RPMI 1640		
None	34	0
Serum	58	1,530
MM-2		
None	28	400
Serum	46	2,100

[a] Plus 6 mM L-cysteine and ascorbic acid; no serum or Cohn III.

[b] Survival at 2 h was virtually unaffected by the absence of serum (see Fig. 3).

[c] Heat-inactivated bovine serum, 10% (vol/vol).

lation was quite consistent, although its magnitude varied from experiment to experiment. Stimulation of attachment at 2 h varied from 40% to sevenfold; serum (or Cohn III) dependence of survival at 10 to 12 h was 2.5-fold to absolute (cf. Table 4 and Fig. 1). The vitamin solution was only slightly stimulatory of attachment and survival in the presence of Cohn III (data not shown). This effect was not considered to be a major one.

The effects of whole serum and Cohn III were compared over a wide range of concentrations (Fig. 1). More trophozoites remained viable with Cohn III at concentrations above 0.05 mg/ml than with serum. Viability was not greatly increased by higher concentrations of either serum or Cohn III. In contrast, the number of organisms attached increased with increasing concentrations of Cohn III, whereas serum appeared to be slightly inhibitory at greater than 5% (Fig. 1A). The activity of Cohn III was not dialyzable and was found to be in a molecular-weight fraction of greater than 10,000. Further fractionation has not yet been attempted.

Fibronection (or cold-insoluble globulin) is a blood and cell surface protein which promotes adhesion of many types of vertebrate cells to a substratum (11). Purified fibronectin did not significantly increase attachment of *G. lamblia* trophozoites (Table 4, experiment 2).

Kinetics of attachment and survival. The rate of attachment was greatest in the first hour in MM-2 as well as in the rich tissue culture medium 199 and the TP-S-1 growth medium base with Cohn III and cysteine plus ascorbic acid (C-A) (Fig. 2). In MM-2, however, the number of attached cells was greatest at 2 h and then declined during the next 8 h, although viability remained high. Stimulation by Cohn III was greatest in MM-2, the most minimal medium. Without C-A, there was no attachment (see Table 2).

The effects of Cohn III and C-A on parasite survival in these three nongrowth media are shown in Fig. 3. Without these additives, the trophozoites died within 3 h. In the absence of C-A, Cohn III promoted parasite survival (for ~10 h) only in MM-2. With C-A, Cohn III increased survival in all media (Fig. 3). The kinetics of survival were similar with serum, except in TP-S-1 medium, parasite growth was observed, whereas with Cohn III, viability declined after 24 h. Evidently, serum contains other factors required for growth. Comparison of Fig. 2 and 3 (data from the same experiment) shows that, in MM-2, attachment was decreased immediately in the absence of Cohn III, but viability (in the presence of reducing agents) was affected only after 4 h.

Physiological factors: temperature. At a reduced temperature (24°C), both the rates of *G. lamblia* attachment and the numbers attached were diminished. At 12°C or below, attachment was not detected (Fig. 4). Parasite viability did not decrease during this period of incubation at the temperatures tested.

Ionic composition and pH. In MM-2, both attachment and survival were greatest at an ionic strength of between 200 and 300 mosmol/kg, and NaCl was better tolerated than KCl (Fig. 5). The osmolality of standard MM-2 was 253 mosmol/kg. Optimal growth was observed at 300 to 360 mosmol/kg in TP-S-1 medium (data not shown). The concentration of divalent ions was reduced by preparing MM-2 without Ca^{2+} or Mg^{2+} and with Cohn III, which had been dialyzed extensively against phosphate-buffered saline. Attachment was decreased only very slightly (<22% [Table 5]) in this medium with or without 1 mM EDTA or EGTA, which would reduce the Mg^{2+} and Ca^{2+} concentrations to 10^{-8} M (1, 17). Viability was not affected, indicating that free Mg^{2+} and Ca^{2+} may not be important in the attachment of *G. lamblia* trophozoites.

Attachment and survival were strongly affected by the pH of MM-2, with sharp optima at pH 6.85 to 7.0 (Fig. 6). The optimum pH for growth in TP-S-1 medium was also 6.9 to 7.0 (data not shown). The effects of pH on attachment and survival in the tissue culture media were not determined. However, the organisms survived and attached in these media (buffered at approxi-

TABLE 4. Effects of serum fractions on attachment and survival of *G. lamblia* trophozoites in MM-2 prepared without serum or Cohn III

Expt and addition[a]	No. of cells attached × 10^3 at 2 h (% control)	No. of survivors: colonies per ml at 9 h (% control)
1		
None	65.0 (59.5)	20 (1.0)
Bovine serum	89.0 (81.4)	1,880 (92.0)
Cohn fraction III[b]	109.3 (100.0)	2,040 (100.0)
Cohn fraction II[c]	82.3 (75.3)	10 (0.5)
Cohn fraction IV[d]	80.4 (73.6)	10 (0.5)
β-Lipoprotein	81.0 (74.0)	20 (1.0)
Fibrinogen	43.4 (39.7)	40 (2.0)
Transferrin	79.5 (72.7)	20 (1.0)
Growth medium	111.1 (102.0)	2,550 (125.0)
2		
None	29.4 (32.6)	570 (20.0)
Cohn fraction III	90.3 (100.0)	2,855 (100.0)
Bovine serum	32.4 (35.9)	980 (34.0)
Cohn fraction V[e]	37.7 (41.7)	252 (8.8)
Human fibronection		
2.5 μg/ml[f]	30.2 (33.0)	720 (25.2)
25 μg/ml	27.4 (30.0)	60 (2.1)

[a] Serum was 10% (vol/vol); fractions were added to 1 mg/ml.
[b] Approximately 50% β-globulins and 25% each α- and γ-globulins.
[c] γ-Globulins.
[d] α-Globulins (crude).
[e] Albumin.
[f] According to the supplier, this preparation promotes spreading of baby hamster kidney cells at concentrations of 1 μg/ml and above.

mately pH 7.2) at least as well as they did in MM-2 at pH 6.9.

Inhibition of attachment. It has been observed (5; Erlandsen et al., J. Cell Biol. **79**:264a, 1978; Feely and Erlandsen, J. Cell Biol. **87**:218a, 1980) that the microfilament inhibitor cytochalasin B and the chemotherapeutic agent quinacrine-hydrochloride (atabrine) inhibited in vitro attach-

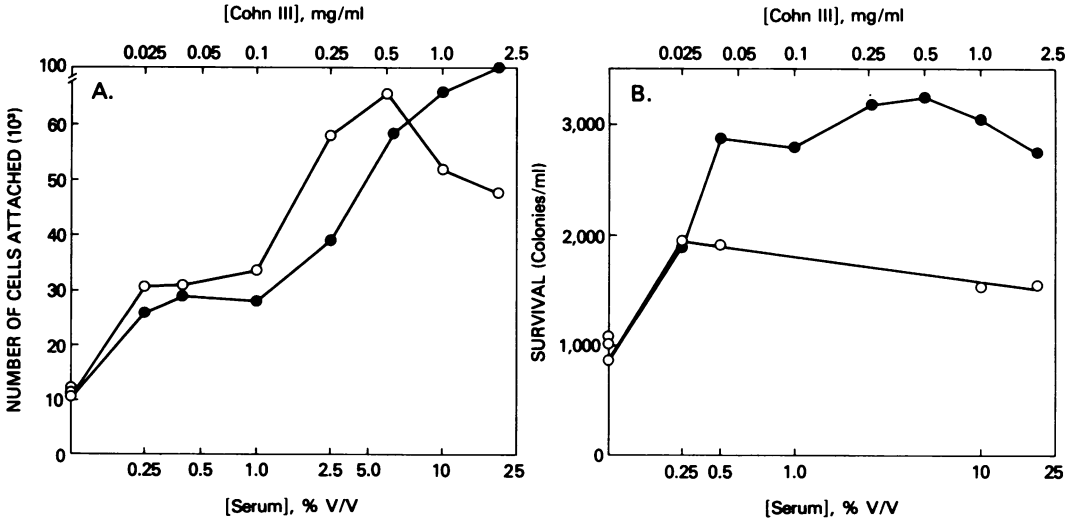

FIG. 1. Attachment and survival of *G. lamblia* trophozoites in the presence of varying concentrations of serum (○) and Cohn III (●). Serum contained approximately 70 mg of protein per ml.

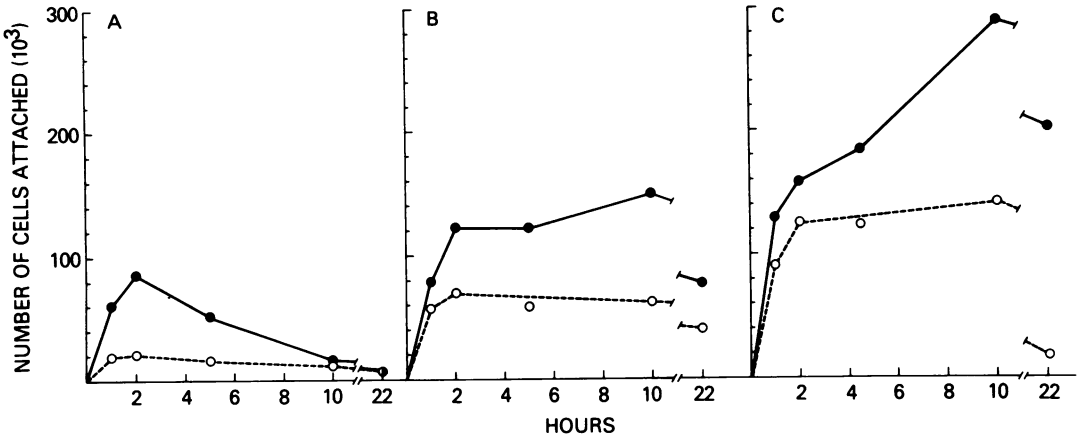

FIG. 2. Kinetics of trophozoite attachment in the presence (●——●) and absence (○------○) of Cohn fraction III. (A) MM-2; (B) medium 199; (C) TP-S-1 medium. All media contained cysteine and ascorbic acid (6 mM each), but no serum. Cohn III was added to a final concentration of 1 mg/ml.

ment of *Giardia* trophozoites which had been isolated from rat small intestines. In contrast, the attachment of cultivated *G. lamblia* trophozoites was not significantly inhibited by cytochalasin B, C, or D (Table 5). Inhibition of attachment of *G. lamblia* trophozoites by quinacrine was observed only at high concentrations which killed the organisms during the 2-h incubation (Table 5).

DISCUSSION

The propensity of *G. lamblia* trophozoites to attach to surfaces is striking. The present studies reveal some of the factors which permit or stimulate in vitro attachment of cultivated *G. lamblia* trophozoites.

The rate of attachment was always most rapid in the first hour of incubation. The initial rates of attachment were similar in the simple maintenance medium (MM-2), the richer medium 199, and the complex growth medium (TP-S-1) containing cysteine, ascorbic acid, and Cohn III. Cell survival for 10 h was also similar in these media. In contrast, the numbers of attached cells after the initial rapid phase of attachment differed; in MM-2, the number of attached cells declined and in medium 199 it remained constant, whereas in TP-S-1, the number of at-

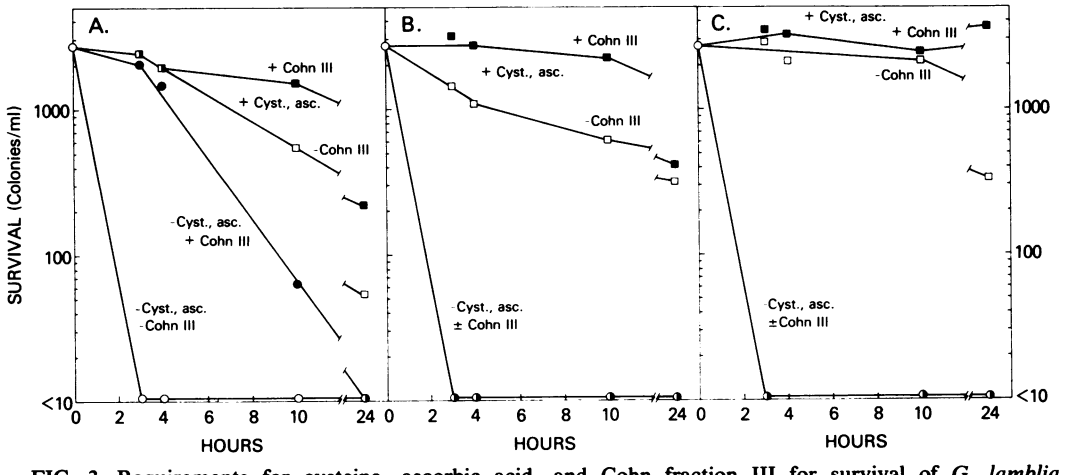

FIG. 3. Requirements for cysteine, ascorbic acid, and Cohn fraction III for survival of *G. lamblia* trophozoites. Effects of Cohn III and cysteine and ascorbic acid (6 mM each) on survival of trophozoites are shown. (A) MM-2; (B) medium 199; (C) TP-S-1 medium. Media prepared without serum, Cohn III, or reducing agents were supplemented as indicated. asc., Ascorbic acid.

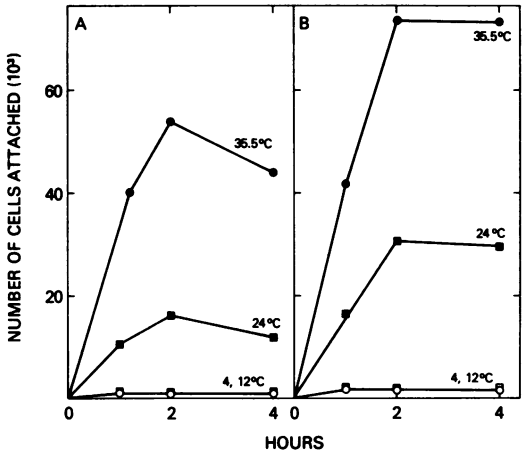

FIG. 4. Effects of incubation temperature and medium on attachment of *G. lamblia* trophozoites. (A) Complete MM-2; (B) medium 199 without serum or $NaHCO_3$, supplemented with Cohn III (1 mg/ml) and C-A.

tached cells increased, but at less than the initial rate. Thus, in less rich media, the trophozoites may become depleted of nutrients necessary for attachment, but not for survival.

The most striking requirement for *G. lamblia* attachment and short-term survival was that of a thiol reducing agent. The requirement was not specific, as six of eight thiol compounds tested, including D-cysteine, were effective. Thiol compounds had earlier been shown to be required for growth of *G. lamblia*; L-cysteine yielded the greatest growth, whereas D-cysteine yielded no growth (9). Evidently, the thiol requirement for growth is more specific than that for attachment. Ascorbic acid, which in the presence of a thiol compound stimulated both growth and attachment, was completely ineffective alone. *G. lamblia* may require either the thiol group or a lower redox potential than that afforded by ascorbic acid.

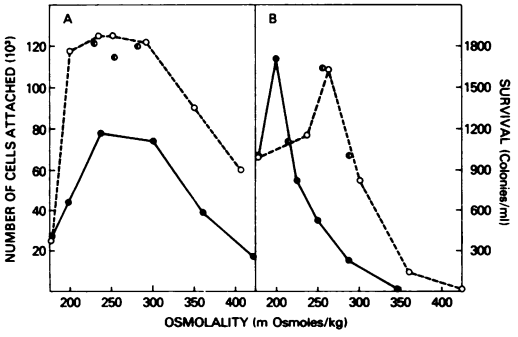

FIG. 5. Effects of ionic composition upon attachment at 2 h (A) and survival at 10 h (B). Incubation was in MM-2 prepared without added NaCl (○) or KCl (●) and supplemented as indicated. The mixture (◐) was 12 mM KCl plus varying concentrations of NaCl.

TABLE 5. Inhibition of attachment

Expt and inhibitor (concn)	% Control[a]	
	Attachment	Survival
I. Quinacrine		
1 μg/ml	114	100
10 μg/ml	85	1
100 μg/ml	22	ND[b]
II.		
Cytochalasin B[c]		
10 μg/ml	97	
25 μg/ml	91	
Cytochalasin C		
10 μg/ml	95	
25 μg/ml	94	
Cytochalasin D		
10 μg/ml	87	
25 μg/ml)	74	
III. No added Mg^{2+} or Ca^{2+}[d]	83	
+ EDTA (1 mM)	83	
+ EGTA (1 mM)	78	

[a] Incubation was for 2 h in MM-2 for both attachment and survival assays.

[b] ND, Not determined.

[c] In preliminary experiments, *G. lamblia* trophozoites were not killed in 2 h by cytochalasin B, C, or D at concentrations of up to 50 μg/ml or by dimethyl sulfoxide (0.4 to 2%) in controls.

[d] Controls contained 8 mM $MgCl_2$ and 2 mM $CaCl_2$. No differences in pH or in survival at 2 h were observed between experimentals and controls in preliminary experiments. These results are not considered significantly different from those obtained with controls.

Entamoeba histolytica, an unrelated aerotolerant anaerobic parasitic protozoan, also required reducing agents for growth and attachment (8). This organism differed from *G. lamblia* in that the maximum growth observed in a complex medium was obtained with either D- or L-cysteine, L-cystine, or ascorbic acid (9). In a minimal maintenance medium, however, D- or L-cysteine and ascorbic acid were absolutely and specifically required for attachment and survival of the amoebae. In contrast to the observations with *G. lamblia*, no other thiol compound was effective (8).

It is not surprising that serum stimulates attachment of *G. lamblia* trophozoites. This organism apparently requires serum for growth (14,

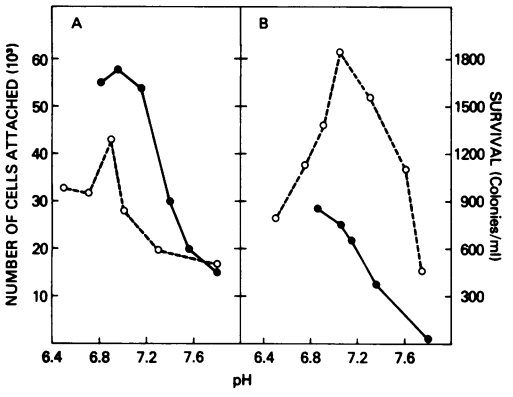

FIG. 6. Effects of pH upon attachment at 2 h (A) and survival at 10 h (B) in MM-2 with 10% serum (●) or 1 mg of Cohn III (○) per ml. MM-2 was prepared with the Tris buffer adjusted to varying pH with HCl. The final pH is shown.

18), and many cultured mammalian cells require serum or serum factors for attachment or growth or both (13). One such factor, fibronectin or cold-insoluble globulin (11), apparently was not required for attachment of *G. lamblia*. The crude Cohn III serum fraction evidently contained the serum components required for attachment and short-term survival. Serum, however, contained additional factors required for *G. lamblia* growth. Attachment of *E. histolytica*, in contrast, was best stimulated by bovine serum albumin (Cohn fraction V) (6).

Cohn III is a crude serum fraction containing approximately 50% β-globulins, 25% α-globulins, and 25% γ-globulins as analyzed by the supplier. A purified preparation of γ-globulins (99%) and a crude preparation of α-globulins (~63% α-globulins with 23% β-globulins and 14% albumin) were not active. It is likely that the active factor is a minor component of the Cohn III fraction (which has not yet been identified). Nonetheless, use of the Cohn III fraction is advantageous, as it is less complex than serum and permits lowering of the protein concentration by at least sevenfold.

Using *Giardia* trophozoites purified from rat small intestines (4), Feely and Erlandsen observed inhibition of attachment to plastic by the microfilament inhibitor cytochalasin B, the chemotherapeutic flavin antagonist quinacrine-hydrochloride (16), or low Ca^{2+} or low temperature (5; Feely and Erlandsen, J. Cell Biol. 87:218a, 1980). The *Giardia* trophozoites used in their studies were of two morphological types and remained viable for 2 to 4 h. These *Giardia* trophozoites have not been cultivated, and viability was determined by dye exclusion.

With cultured *G. lamblia* trophozoites, we observed no significant inhibition of attachment by cytochalasin B, C, or D or by low Ca^{2+}. Quinacrine-hydrochloride inhibited attachment only at concentrations which killed the organisms during incubation. It is likely that the observed differences were due to the different *Giardia* species and methods employed. We have observed motile, normal-appearing *G. lamblia* trophozoites which were irreversibly damaged so they could not multiply into colonies.

Attachment and short-term survival were also strongly dependent upon pH and ionic composition. Local variations in pH and ionic composition as well as redox potential in the small intestine of the host may influence attachment of *G. lamblia* trophozoites to the intestinal epithelium and mediate differentiation of the motile trophozoites into the cyst form lower in the digestive tract.

Availability of relatively simple media (MM-2 199 with C-A, and Cohn III) which allow attachment and survival of *G. lamblia* trophozoites permits studies of interactions of *G. lamblia* with mammalian cells, lectins, and antibody and complement (studies currently in progress).

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