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Exposure to Low Oxygen Tension and Increased Osmolarity Enhance the Ability of *Mycobacterium avium* To Enter Intestinal Epithelial (HT-29) Cells

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Current evidence indicates that *Mycobacterium avium* infection in patients with AIDS is acquired mostly through the gastrointestinal (GI) tract and that *M. avium* binds to and invades GI mucosal cells in vitro. Since *M. avium* is exposed to specific environmental conditions in the GI tract such as changes in pH, low oxygen (O_2) tension, increased osmolarity, and low concentration of iron, we investigated the effects of these conditions on the bacterium's ability to enter HT-29 intestinal cells. *M. avium* 101 (serovar 1) was cultured in 7H9 broth and then exposed to pH 4.5 to 8.0, low O_2 tension, 0.1 to 0.3 M dextrose, and absence of iron for 2 h. After washing, bacteria (10^7/ml) were used in the invasion assay. Confluent HT-29 cells were exposed to 10^6 bacteria for 1 h and then treated with amikacin (200 μg/ml) for 2 h to selectively kill extracellular but not intracellular *M. avium*. The supernatant was then removed, the monolayer was lysed, and the lysate was plated onto 7H10 agar plates. While exposure to acidic and basic pHs, as well as iron-free medium, had no significant effect on *M. avium* invasion of intestinal epithelial cells, low O_2 tension and increased osmolarity enhanced invasion 11- and 9-fold, respectively, compared with the control. Exposure of *M. avium* to the combination of low O_2 concentration and hyperosmolarity resulted in an approximate 10- to 15-fold increase in penetration of HT-29 cells. Hyperosmolarity and low O_2 tension induced the invasive *M. avium* phenotype and can be useful for the identification of genes associated with *M. avium* invasion of intestinal mucosa.

Mycobacterium avium is primarily a bird pathogen that can be found free-living in the environment and is commonly isolated from water (12, 17, 18, 28). In contrast to non-AIDS patients, in whom *M. avium* is often associated with pulmonary disease that resembles tuberculosis, in patients with AIDS, *M. avium* colonizes the intestine and causes disseminated disease (15, 16, 22).

Following ingestion by both in mice and humans, *M. avium* is found within the intestinal mucosa and submucosa from the stomach to the colon (3, 16). However, in mice the great majority of organisms are encountered in the terminal ileum and ascending colon (3). Previous studies in vitro had established that *M. avium* strains are capable of binding and invading intestinal epithelial cells (4, 5, 26). In addition, it was demonstrated that the invasion process was dependent on both the growth phase of the bacterium and the temperature (5). At temperatures that reproduce the temperature outside the host (23 and 30°C), *M. avium* was significantly less efficient in invading intestinal cells than at 37°C (temperature inside the host), which suggests that genes associated with virulence may be regulated by temperature and possibly other environmental stimuli to facilitate host-pathogen interaction.

Similar observations have been reported for *Yersinia pseudotuberculosis* (21), *Vibrio cholerae* (31), and *Salmonella* spp. (24, 25). *Y. pseudotuberculosis*, for example, upregulates the expression of the *inv* gene (associated with invasion of epithelial cells) when exposed to acid pH (21). Likewise, the *Salmonella* invasive phenotype is induced by low concentrations of oxygen in the environment (25).

M. avium causes infection in AIDS patients primarily after invasion of the intestinal mucosa (34). Therefore, it was of interest to investigate whether environmental conditions encountered within the intestinal tract, such as pH, low oxygen tension, increased osmolarity, and low iron concentration, would regulate the *M. avium* gene(s) associated with entry into the intestinal mucosa.

MATERIALS AND METHODS

Mycobacterium. *M. avium* 101 (serovar 1) was used for the experiments. Previous work determined that this is an invasive strain of *M. avium* (5). It was isolated from the blood of a patient with AIDS, and its ability to cause disseminated infection in mice has been established in this as well as other laboratories. For the assays, *M. avium* was cultured in Middlebrook 7H10 agar for 10 days, and isolated transparent colonies were washed and resuspended in Middlebrook 7H9 broth for 5 days (logarithmic phase). Prior to the assays, bacteria were washed in Hanks' buffered salt solution (HBSS) and passed through an 18-gauge needle 10 times. The suspension was then placed in a 15-ml polystyrene tube and vortex agitated for 2 min. The tube was placed to rest for 5 min, after which the top 1 ml was removed and used as a source of bacteria. The bacterial inoculum was stained by the Ziehl-Neelson technique and observed under a light microscope. Only an inoculum with dispersed bacteria was used in the assays. The bacterial inoculum was also plated onto 7H10 agar for quantitation. Bacterial viability in the inoculum was determined to be between 90 and 92% by using the LIVE-DEAD assay (Molecular Probes, Portland, Oreg.) as previously reported (1).

Cells. Colon carcinoma cell line HT-29, a well-differentiated cell line with marked characteristics of human intestinal cells, was purchased from the American Type Culture Collection (Rockville, Md.) and maintained in McCoy 5A medium without glucose (Gibco Laboratories, Detroit, Mich.) supplemented with 1% galactose, 2 mM L-glutamine, and 5% fetal bovine serum (Sigma Chemical Co., St. Louis, Mo.). For the assays, 10^5 HT-29 cells were seeded in each well and left to grow for 4 days to 80% confluence in a 24-well tissue culture plate. The cell line was used between passages 45 and 56 in all assays.

Invasion assay. The invasion assay was carried out as previously described (5). It was adapted for mycobacteria from a method previously described by Isberg and Falkow (20). Briefly, prior to the assay, the culture medium of the monolayers was removed and replaced with prewarmed (37°C) culture medium. Bacterial samples were added, and the monolayers were incubated at 37°C in a 5% CO_2 incubator for 1 h. Assays were terminated by replacing the overlying medium with 1 ml of tissue culture medium supplemented with 200 μg of amikacin

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TABLE 1. Influence of the time of exposure to different conditions on the ability of *M. avium* to invade HT-29 cells

Time of exposure (h)[a]	% of initial inoculum[b] (mean ± SD)			
	Standard conditions	pH 5.0	Anaerobiosis	Hyperosmolarity (0.3 M)
0	0.53 ± 0.08	0.51 ± 0.09	0.53 ± 0.07	0.51 ± 0.08
1	0.53 ± 0.09	0.50 ± 0.07	1.77 ± 0.08[c,d]	1.65 ± 0.06[c,d]
2	0.52 ± 0.08	0.48 ± 0.06	8.9 ± 0.9[c,d]	8.3 ± 0.9[c,d]
3	0.54 ± 0.09	0.50 ± 0.1	9.1 ± 1.1[c,d]	8.5 ± 0.7[c,d]
4	0.57 ± 0.07	0.52 ± 0.09	9.0 ± 1.2[c,d]	8.5 ± 1.0[c,d]
24	0.55 ± 0.05	0.41 ± 0.06	8.2 ± 1.4[c]	8.3 ± 0.9[c]

[a] Bacteria were incubated at 37°C. Invasion was measured after 1 h of incubation.
[b] Initial inoculum, approximately 10^6 bacteria. Conditions were achieved as described in Materials and methods.
[c] $P < 0.001$ compared to baseline.
[d] $P < 0.05$ compared to standard conditions control at the same time point.

per ml. Amikacin, an aminoglycoside antibiotic, at this concentration kills extracellular *M. avium*, while intracellular bacteria remain viable (5). After the incubation with amikacin for 2 h at 37°C in 5% CO_2 atmosphere, the monolayers were washed twice with HBSS and the viable intracellular bacteria were released by incubation with 0.5 ml of 0.1% Triton X-100 (Sigma) in sterile water for 10 min. Samples were vigorously mixed with 0.5 ml of Middlebrook 7H9 broth and harvested. Viable bacteria were quantitated by plating for CFU onto Middlebrook 7H10 agar.

Effect of environmental conditions. In an attempt to reproduce the conditions encountered by mycobacteria in the intestines in vivo, we tested the effects of a number of environmental conditions, both individually and in combination, on the ability of *M. avium* to invade HT-29 intestinal cells.

Bacteria were washed, resuspended in 7H9 broth without oleic acid, albumin, dextrose, and catalase (OADC), and then exposed to different conditions for 1 to 24 h at 37°C. Usual laboratory conditions (controls) were defined at 37°C, 20% O_2, and pH 7.4. Different pHs were established by using 0.1 N HCl and 0.1 N NaOH.

The effect of iron was tested aerobically in complete 7H9 as well as 7H10 agar medium (pH 7.4) by supplementing the medium with $FeCl_3$ or by removing iron with 100 μM 2,2-dipyridyl (Sigma).

The effect of pH was tested aerobically and anaerobically in Middlebrook 7H9 broth. The pH was maintained by supplementing 7H9 broth with the following sulfonate buffers with appropriate pK_a values: 7H9 (pKa 6.5), TAPS [tris(hydroxymethyl)methyl-aminopropanesulfonic acid; pKa 8.4), and citrate buffer (pKa 4.5).

The effect of increased osmolarity was tested aerobically and anaerobically in complete 7H9 medium supplemented with dextrose at equimolar concentrations (0.1, 0.2, and 0.3 M).

Oxygen-deficient and anaerobic media were prepared by the methods described by Lee and Falkow (24) and Cotter and Gunsalus (10), respectively.

The number of bacteria in the inoculum after exposure to the different conditions as well as the degree of dispersion of the suspension were examined. No condition was shown to cause bacterial clumping.

RNA synthesis. RNA synthesis was determined by the incorporation of [^{3}H]uracil. *M. avium* 101 (10^7 bacteria) was incubated at pH 5.0 in hyperosmolar (0.3 M) or anaerobic conditions for 2 h in 7H9 medium without OADC and in the presence of 1 μCi of [^{3}H]uracil (specific activity, 51 Ci/mmol). After 2 h, bacteria were washed three times in HBSS and the incorporation of [^{3}H]uracil was determined in a beta counter as previously described (7). A control, in which unlabeled uracil was added prior to the addition to [^{3}H]uracil, was run in parallel.

Statistics. All experiments were carried out in triplicate and repeated three times. Statistical analysis was done by using analysis of variance and Student's *t* test.

RESULTS

Influence of exposure to conditions resembling environmental cues. To determine whether incubation of *M. avium* under conditions that resemble the intestinal environment affects the bacterium's ability to invade intestinal epithelial cells, *M. avium* was exposed to acidic pH, anaerobiosis, or hyperosmolarity (0.3 M) for periods varying from 0 to 24 h. Subsequently bacteria were recovered after washing at 4°C and incubated with HT-29 intestinal epithelial cells for 1 h. As shown in Table 1, incubation in the presence of increased osmolarity or low tension of oxygen but not acidic pH was associated with a significant increase in invasion (Table 1, Table 2, Fig. 1, and Fig. 2). The peak of invasion under both conditions was achieved after 2 h of incubation, while a decline was seen at 24 h. We believe that the level of invasion was lower than the average of 3% ± 1% at 1 h (4, 5) because bacteria were exposed to a temperature of 4°C during the washing.

TABLE 2. Quantitation of intracellular *M. avium* 101 after invasion of HT-29 cells

Conditions preexposed to[a]	No. of bacteria/cell (mean ± SD)	No. of infected cells/50 cells counted[b]
None (control)	1 ± 1	3
Anaerobiosis	6 ± 3	36
High osmolarity (0.3 M)	5 ± 3	38
Acid pH (5.0)	1 ± 1	3

[a] As described in Materials and Methods.
[b] Determined by electron microscopy.

Effect of exposure to low oxygen tension on invasion. As shown in Table 3 and Fig. 1, *M. avium* cells incubated for 2 h in the presence of 20, 10, 5, and 0% oxygen showed different abilities to invade HT-29 intestinal cells. A direct relationship was observed between decreasing in oxygen tension and efficiency of invasion. Uptake of *M. avium* exposed to anaerobiosis for 2 h was approximately ninefold greater than the uptake of *M. avium* incubated in the presence of atmospheric oxygen tension (20%) ($P < 0.001$).

Effect of exposure to increased osmolarity on invasion. Hyperosmolarity was determined to induce invasion by *M. avium*. Table 3 and Fig. 1 show that the increase in the ability to enter HT-29 intestinal cells was directly correlated with previous incubation under increased osmolarity. Incubation of *M. avium* in a medium with an osmolarity of 0.3 M for 2 h resulted in an eight- to ninefold increase in uptake.

Effect of exposure to the combination of hyperosmolarity and anaerobiosis. Since mycobacteria are exposed to a variety of environmental conditions in the intestinal lumen, we carried out assays to examine the combined effects of hyperosmolarity and anaerobiosis. *M. avium* was incubated with exposure to anaerobiosis or hyperosmolarity for 2 h, sequentially to both for 1 h each (total 2 h), or simultaneously to both for 2 h. As shown in Table 4, only exposure to the combination of anaerobiosis and hyperosmolarity resulted in a significant increase in uptake compared to either one alone.

Effect of exposure to the combination of acid pH with either hyperosmolarity or anaerobiosis. Incubation of *M. avium* in pH 5.0 and hyperosmolarity (0.3 M) did not result in an increase in bacteria uptake compared with exposure to hyperosmolarity alone. Incubation under hyperosmolar conditions for 1 h prior to exposure to HT-29 cells resulted in a small but significant increase in invasion, while incubation in hyperosmolar conditions for 1 h followed by incubation at acidic pH was not associated with increased ability to invade HT-29 cells (Table 5).

Table 6 shows the results of similar studies using anaerobiosis and acidic pH as environmental cues. Our observation was that incubation of *M. avium* under anaerobiosis at an acidic pH had an effect on *M. avium* uptake by HT-29 cells similar to that of hyperosmolarity and acidic pH. Acidic pH does not seem to influence the bacterial response to either anaerobiosis or hyperosmolarity.

Effect of low iron concentrations on *M. avium*. The availability of iron can be low in the intestinal tract (29). However, *M.*

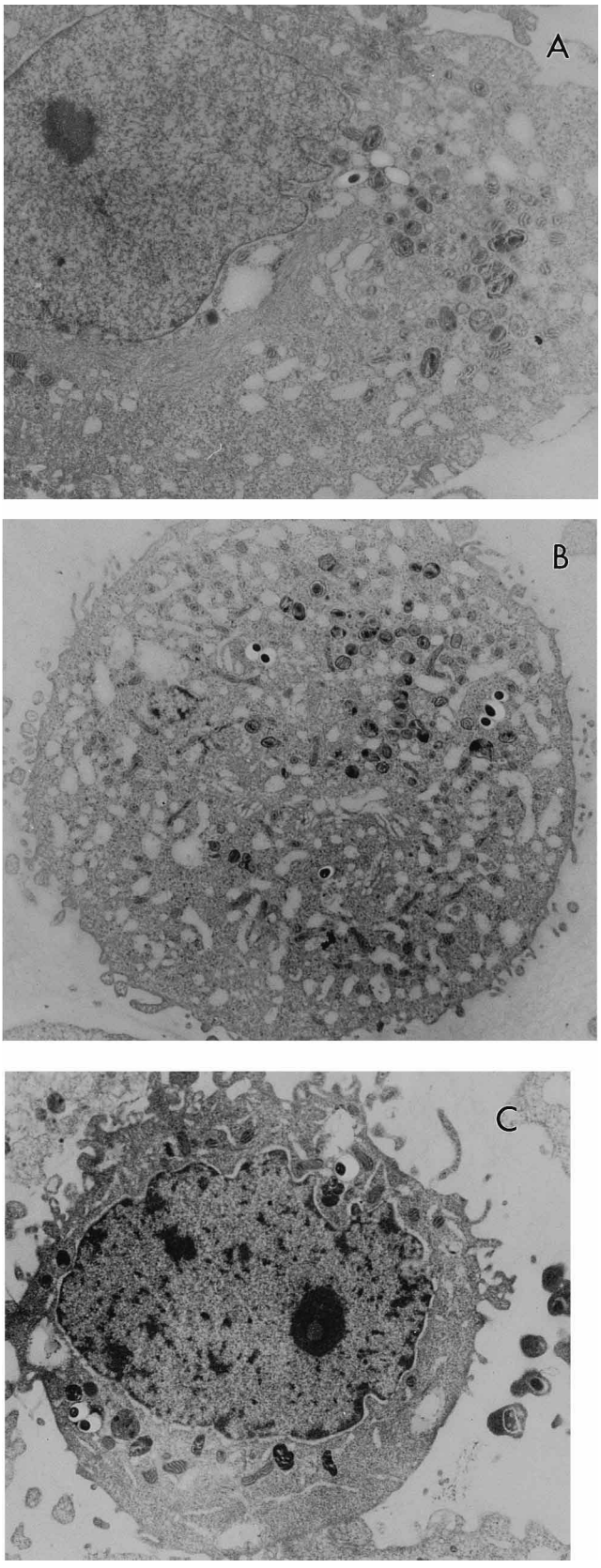

FIG. 1. Electron micrographs showing HT-29 cells after 1 h of incubation with control bacteria (A) and *M. avium* incubated for 2 h in the presence of hyperosmolar conditions (B) and anaerobiosis (C). Magnification, ×9,548.

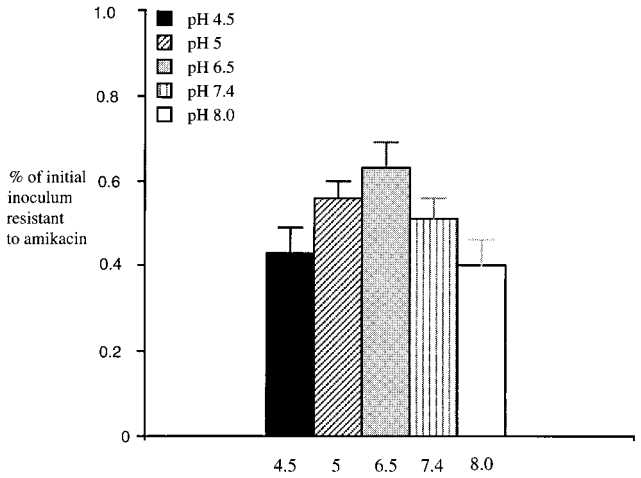

FIG. 2. Effects of different pHs on the ability of *M. avium* to invade HT-29 intestinal cells.

avium cultured for 2 h in the absence of iron did not differ in its ability to enter HT-29 cells compared with *M. avium* grown in medium with iron (using 80% confluent monolayers [data not shown]).

RNA synthesis. Because *M. avium* responded to both hyperosmolar and anaerobic conditions with increased ability to enter intestinal epithelial cells (Tables 5 and 6), we examined *M. avium* RNA synthesis following exposure to acidic pH, hyperosmolarity, or anaerobiosis. As shown in Fig. 3, incubation of *M. avium* in the presence of acidic pH, hyperosmolarity, or low O_2 tension for 2 and 4 h led to a significant incorporation of [^{3}H]uracil. The fact that exposure to acidic pH is not associated with increased invasion of HT-29 cells but resulted in rapid incorporation of [^{3}H]uracil probably means that acidic pH triggers expression of genes not linked with entry of *M. avium* into epithelial cells.

Effect of inhibition of protein synthesis on anaerobiosis- and hyperosmolarity-mediated increase in *M. avium* uptake by HT-29 cells. In an attempt to determine whether the effects of conditions resembling environmental cues on *M. avium* uptake

TABLE 3. Effect of exposure to low tension of oxygen and increased osmolarity on the ability of *M. avium* to invade HT-29 intestinal cells

Environmental conditions[a]	% of initial inoculum amikacin resistant[b] (mean ± SD)
Control O_2 (20% O_2 tension)	0.53 ± 0.09
Low O_2, at O_2 tension of:	
10%	3.7 ± 0.08[c]
5%	6.9 ± 0.6[c]
0%	9.3 ± 1.1[c]
Control osmolarity	0.52 ± 0.08
Increased osmolarity	
0.1 M	3.1 ± 0.9[c]
0.2 M	6.7 ± 1.0[c]
0.3 M	8.6 ± 0.6[c]

[a] *M. avium* was cultured in Middlebrook 7H9 broth for 5 days and was exposed to the indicated conditions for 2 h.

[b] The invasion assay was carried out as described in Materials and Methods, and then extracellular bacteria were exposed to amikacin (200 μg/ml) for 2 h. Initial inoculum, (1 ± 0.3) × 10^6 bacteria.

[c] $P < 0.01$ compared with control.

TABLE 4. Effect of exposure to combination of hyperosmolarity and anaerobiosis on *M. avium* invasion of HT-29 cells

Conditions (duration of exposure)	% of initial inoculum amikacin resistant[a] (mean ± SD)
Standard	0.51 ± 0.07
Hyperosmolarity, 0.3 M (2 h)	8.7 ± 0.6[b]
Anaerobiosis (2 h)	9.2 ± 0.9[b]
Hyperosmolarity (1 h) + anaerobiosis (1 h)[c]	10.04 ± 1.1[b]
Anaerobiosis (1 h) + hyperosmolarity (1 h)	9.6 ± 1.2[b]
Anaerobiosis + hyperosmolarity (2 h)[d]	13.5 ± 2.1[e]

[a] Percentage of the initial inoculum that was intracellular.
[b] $P < 0.05$ compared with standard control.
[c] *M. avium* was exposed to hyperosmolarity for 1 h and then anaerobiosis for an extra hour.
[d] *M. avium* was exposed to the combination of hyperosmolarity and anaerobiosis for 2 h.
[e] $P < 0.05$ compared to both anaerobiosis and hyperosmolarity alone.

by intestinal epithelial cells were due to induction of a phenotypic change in the bacterium, we carried out studies in which *M. avium* was incubated under acidic pH, hyperosmolarity, or anaerobiosis for 2 h in the presence or absence of amikacin at 12 μg/ml (subinhibitory concentration that inhibits protein synthesis). As shown in Fig. 4, incubation in the presence of amikacin significantly suppressed the increase in invasion observed when *M. avium* is exposed to hyperosmolar and anaerobiosis conditions.

DISCUSSION

The expression of a number of bacterial genes is regulated by environmental conditions. For example, oxygen tension regulates the expression of the *ail* gene, and low pH affects the regulation of the *inv* gene in *Y. enterocolitica* (32, 33). Iron regulates the expression of diphtheria cytotoxin (11). Temperature affects invasion of *Shigella flexneri* (27), *Y. tuberculosis* (19), *Bordetella pertussis* (30), and *M. avium* (5) into epithelial cells. Osmolarity has been noted to be an environmental signal controlling the expression of genes associated with virulence of several organisms, among them the *toxR* gene of *V. cholerae* (31) and the *ompR* genes of *Salmonella* and *Shigella* species (8, 9).

Present evidence supports the gastrointestinal tract as the most important route of *M. avium* infection in AIDS patients. Organisms found in the intestinal lumen, and not the respiratory tract, have been associated with disseminated disease by

TABLE 5. Effect of exposure to combination of acid pH and hyperosmolarity on *M. avium* invasion of HT-29 cells

Conditions (duration of exposure)	% of initial inoculum amikacin resistant[a] (mean ± SD)
Standard	0.51 ± 0.07
pH 5.0 (2 h)	0.56 ± 0.08
Hyperosmolarity, 0.03 M (2 h)	8.9 ± 0.9[b]
pH 5.0 (1 h) + hyperosmolarity (1 h)[c]	2.7 ± 0.3[b]
Hyperosmolarity (1 h) + pH 5.0 (1 h)	0.59 ± 0.07
Hyperosmolarity + pH 5.0 (2 h)[d]	9.2 ± 1.2[b]

[a] Percentage of the initial inoculum that was intracellular.
[b] $P < 0.05$ compared with standard control.
[c] *M. avium* was exposed to acid pH for 1 h and then to hyperosmolarity for another hour.
[d] *M. avium* was exposed to the combination of hyperosmolarity and acid pH for 2 h.

TABLE 6. Effect of exposure to the combination of acid pH and anaerobiosis of *M. avium* invasion of HT-29 cells

Conditions (duration of exposure)	% of initial inoculum amikacin resistant[a] (mean ± SD)
Standard	0.51 ± 0.09
pH 5.0 (2 h)	0.53 ± 0.06
Anaerobiosis (2 h)	8.9 ± 1.1[b]
pH 5.0 (1 h) + anaerobiosis (1 h)[c]	1.9 ± 0.5[b]
Anaerobiosis (1 h) + pH 5.0 (1 h)	0.58 ± 0.06
Anaerobiosis + pH 5.0 (2 h)[d]	9.3 ± 1.6[b]

[a] Percentage of the initial inoculum that was intracellular.
[b] $P < 0.05$ compared with standard control.
[c] *M. avium* was exposed to pH 5.0 for 1 h and then to anaerobiosis for another hour.
[d] *M. avium* was exposed to the combination of hyperosmolarity and acid pH for 2 h.

using DNA fingerprinting techniques (36). In addition, biopsies of the intestinal tracts of AIDS patients have documented a large "leprosy-like" number of *M. avium* organisms in the lamina propria of the mucosa (18, 22). Furthermore, studies in which healthy mice were given a number of AIDS-related strains of *M. avium* have shown that the bacterium can penetrate intact intestinal mucosa and cause disseminated disease in 100% of either immunocompetent or immunocompromised mice (3).

M. avium ingested by a host will transiently encounter a reduced pH in the stomach and acid and basic pH in the intestines. Osmolarity in the intestines is high, with a high NaCl concentration (0.31 OsM). In addition, the terminal ileum, one of the sites of preference for *M. avium* invasion (3), is normally anaerobic (33). Therefore, the results of our study demonstrated that *M. avium* is well adapted to the environmental conditions encountered within the intestines and that both hyperosmolarity and anaerobiosis significantly increase the efficiency of *M. avium* to enter HT-29 cells in culture. In contrast, conditions such as acidic or basic pH and low iron have no impact on *M. avium* invasion. It was also demonstrated that acidic pH does not change the *M. avium* response to either hyperosmolarity or anaerobiosis.

In previous studies, we have shown that temperature influences *M. avium* interaction with epithelial cells (3). However, it was determined that temperature (37°C) by itself was re-

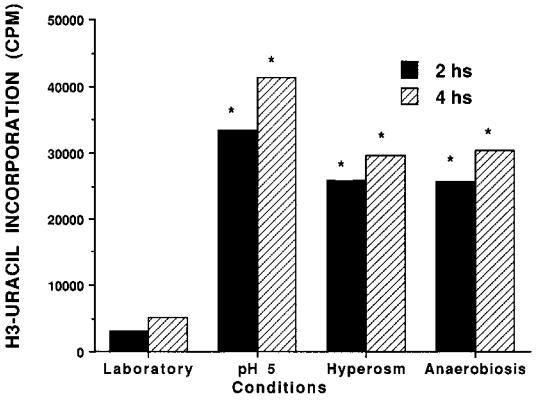

FIG. 3. RNA synthesis as measured by incorporation of [^{3}H]uracil following exposure to acidic pH, anaerobiosis, or hyperosmolarity (hyperosm), for 2 and 4 h, in comparison with bacteria maintained under standard conditions. *, statistically significant compared with control.

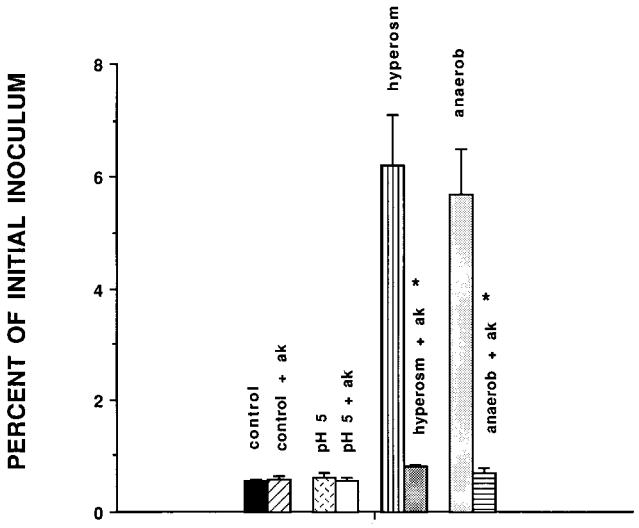

FIG. 4. Effect on subinhibitory concentration of amikacin on the hyperosmolarity- or anaerobiosis-induced increase in the ability of *M. avium* to enter HT-29 cells. Bacteria were incubated in the presence of standard conditions, acidic pH, hyperosmolarity (hyperosm), and anaerobiosis (anaerob) for 2 h in the presence or absence of 12 μg of amikacin (ak) per ml. *, statistically significant, presence versus absence of amikacin.

sponsible for less than 40% of the effects of both hyperosmolarity and anaerobiosis on *M. avium* uptake (data not shown).

The regulation of the invasive phenotype by conditions such as hyperosmolarity and anaerobiosis is reversible, and the augmented ability to invade intestinal epithelial cells associated with them can be inhibited by amikacin in a subinhibitory concentration. This finding, added to the observation that RNA synthesis is induced in *M. avium* exposed to hyperosmolarity and anaerobiosis, suggests that the increase in the efficiency of invasion is due to the synthesis and expression of proteins (or glycoproteins) associated with entry. Interestingly, the evidence that protein synthesis appears to occur very promptly in *M. avium* supports the concept that a pathogen must be able to adapt rapidly to the environment in order to survive, even though the duplication time of *M. avium* is long. We are currently undertaking studies to identify proteins related to the invasive phenotype.

The sigma factor encoded by *rpoS* regulates the expression of several genes needed to tolerate extreme environmental conditions (23). Homologs of the *rpoS* gene have been identified in *S. flexneri* (33), *Salmonella typhimurium* (13), *Yersinia enterocolitica* (33), and more recently *Mycobacterium tuberculosis* (37). We have identified the *M. avium* homolog of the *rpoS* gene (34a), and investigation is under way to determine whether expression of *rpoS* is required under the conditions associated with increased binding and invasion of *M. avium* to epithelial cells.

M. avium binding to and invasion of HT-29 cells can be inhibited by DNA gyrase inhibitors both in vitro and in vivo (6). Alteration in DNA supercoiling has been suggested as the mechanism to explain how osmolarity regulates invasion of *Salmonella typhimurium* through the expression of *invA* (14). However, the role of supercoiling in regulating the expression of genes in *M. avium* is unknown.

Finally, there is accumulating evidence that stress at the level of starvation, pH, and heat shock may control the expression of virulence genes (29). For example, the expression of listeriolysin, a protein involved in intracellular virulence of *Listeria monocytogenes*, is induced by heat shock and oxidative stress (35). Although some of the proteins expressed by *M. avium* upon entry into epithelial cells are heat shock proteins (2), we have no evidence to link the increase of invasion under both hyperosmolarity and anaerobiosis to the *M. avium* response to stress. In addition, the failure of two stress factors, acidic pH and depletion of iron, to induce the expression of invasive phenotype supports the conclusion that the correlation (when it exists) is not always exclusive.

In summary, we have determined that hyperosmolarity and anaerobiosis are environmental signals for optimal entry of *M. avium* to intestinal epithelial cells. Future studies will determine whether the ability to respond to environmental cues can distinguish between *M. avium* strains that can cause disease and those that do not.

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