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NOTES

Intracellular pH of Acid-Tolerant Ruminal Bacteria

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Acid-tolerant ruminal bacteria (*Bacteroides ruminicola* B_14, *Selenomonas ruminantium* HD_4, *Streptococcus bovis* JB1, *Megasphaera elsdenii* B159, and strain F) allowed their intracellular pH to decline as a function of extracellular pH and did not generate a large pH gradient across the cell membrane until the extracellular pH was low (<5.2). This decline in intracellular pH prevented an accumulation of volatile fatty acid anions inside the cells.

For many years it was assumed that all bacteria maintained a near-neutral intracellular pH (8), but recent work has indicated that some acid-tolerant bacteria let intracellular pH decrease as a function of extracellular pH (5, 6, 14). This difference in intracellular pH regulation has been a curiosity. Kashket (6) argued that each organism has a metabolic scheme with a particular pH threshold and that growth can only occur when the intracellular pH is above this value. This argument cannot be disputed, but more subtle questions arise. Why don't all bacteria maintain the same intracellular pH? Can a decrease in intracellular pH be advantageous?

Short-chain volatile fatty acids are often an end product of anaerobic fermentations, but many bacteria (e.g., *Escherichia coli*) are unable to tolerate these acids when the pH is acidic (3, 15–17). With the advent of the chemiosmotic theory, the idea that volatile fatty acids could act as uncouplers became fashionable (1, 7, 15–17). The "uncoupling" theory, however, did not explain why some bacteria tolerated volatile fatty acids even when the pH was low. In the early 1950s, Rogosa et al. noted that lactobacilli grew in the presence of acetate at pH 5.2 (10).

Recent work with *Streptococcus bovis* indicated that acetate was not acting as an uncoupler (12). High concentrations (100 mM) of acetate had little effect on growth rate or proton motive force even at low pH (5.2 or lower), and the addition of large amounts (as much as 360 mM) of acetate to a pH-controlled (5.2) chemostat had little effect on the efficiency of ATP utilization for growth (Y_{ATP}). The resistance of *S. bovis* to acetate at low pH was correlated with its ability to let pH decrease as a function of extracellular pH (12). Previous work had indicated that *Bacteroides ruminicola* B_14, *Selenomonas ruminantium* HD_4, *Megasphaera elsdenii* B159, and strain F were able to grow at lower pH values than other ruminal bacteria (2, 13), but there was no information regarding intracellular pH.

The bacteria were grown anaerobically (500-ml batch cultures, continuously purged with O_2-free carbon dioxide) in basal medium containing the following (per liter): 292 mg of K_2HPO_4, 292 mg of KH_2PO_4, 1,200 mg of NH_4SO_4, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4,000 mg of Na_2CO_3, 600 mg of cysteine hydrochloride, 0.5 g of yeast extract, 1.0 g of Trypticase (BBL Laboratories, Cockeysville, Md.), 28.3 mmol of acetate, 8.1 mmol of propionate, 3.4 mmol of butyrate, and 1 mmol each of isobutyrate, isovalerate, 2-methyl butyrate, and valerate. Sterile glucose (30 mM) was added to the medium after the medium was autoclaved. Strain F cannot use glucose as an energy source (2). In this case, the Trypticase was increased to 15 g/liter. pH was decreased by the addition of concentrated HCl, and growth was monitored by the increase in optical density.

Internal pH was determined by an acid distribution method (14). Growing cultures (0.5 to 0.8 mg of protein per ml) were incubated with [7-^{14}C]benzoate (1.0 μCi, 21.8 μCi/umol), [2-^{14}C]acetate (10 μCi, 55 μCi/umol), [U-^{14}C]taurine (1.0 μCi, 115 μCi/μmol), or ^3H_2O (4.0 μCi, 3.6 μCi/umol)) for 5 min and then centrifuged through silicon oil (equal-parts mixture of Dexter Hysol 550 and 556; Hysol Co., Olean, N.Y.) in a microcentrifuge (13,000 × *g*, 5 min). Supernatant samples (20 μl) were removed, and bottoms of tubes containing cell pellets were removed with dog nail clippers after freezing. Pellets and supernatants were dissolved in aqueous compatible scintillation fluid. The internal volume (3.3 to 4.6 μl/mg of protein) was estimated from the difference between ^3H_2O and [^{14}C]taurine. *M. elsdenii* B159 cells bound taurine, and in this case [^{14}C]xylose was used as the extracellular marker. Radioactive materials were obtained from Amersham Corp., Arlington Heights, Ill.

When the pH of an *S. bovis* JB1 culture was decreased, there was a nearly linear decrease in intracellular pH, and growth was observed when the intracellular pH was as low as 5.4 (Fig. 1a). *Selenomonas ruminantium* HD_4 also grew at low pH values, and once again the decline in intracellular pH was greater than the increase in the pH gradient (ΔpH) across the cell membrane (Fig. 1b). Strain F, a monensin-sensitive, amino-acid-fermenting, ruminal bacterium (2), and *M. elsdenii* B159 were unable to grow at very low pH values, but even these bacteria allowed considerable acidification of the cytoplasm before growth ceased. Previous work indicated that *B. ruminicola* B_14 was more acid resistant than strain GA33 (13), and B_14 let intracellular pH decrease to a greater extent than GA33 before growth was inhibited.

Although anion accumulation (e.g., benzoate$^-$) has provided a basis for intracellular pH measurements (5), the effect of volatile fatty acid anions on bacteria has largely

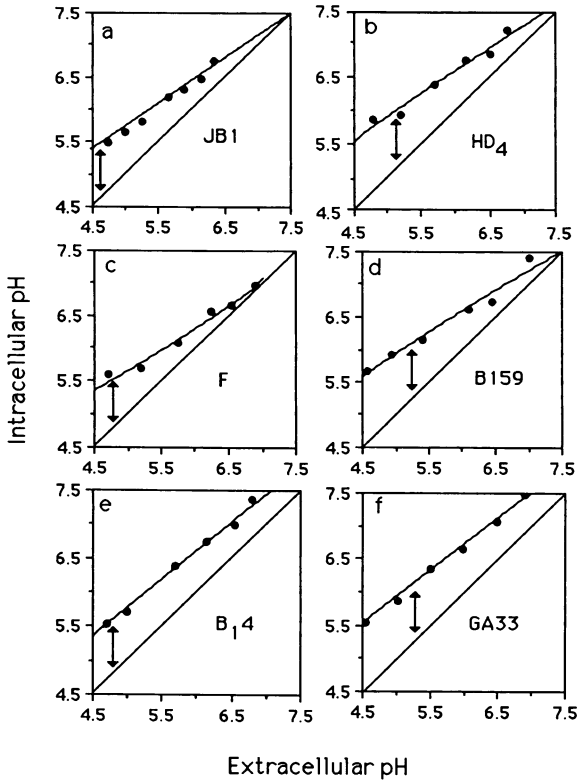

FIG. 1. Effect of extracellular pH on intracellular pH of (a) *S. bovis* JB1, (b) *Selenomonas ruminantium* HD_4, (c) strain F, (d) *M. elsdenii* B159, (e) *B. ruminicola* B_14, and (f) *B. ruminicola* GA33. The arrow shows the pH at which the culture stopped growing. Extracellular pH was decreased by the addition of concentrated HCl.

been ignored (12). In these experiments, the extracellular volatile fatty acid concentration was approximately 50 mM, and all six of the ruminal bacteria stopped growing when the ΔpH was >0.75 U (Fig. 1). On the basis of the Henderson-Hasselbalch equation, a ΔpH of 0.75 will create a sixfold concentration gradient of anion across the cell membrane. *E. coli* maintains an intracellular pH of 7.6 across a wide range of extracellular pH, and there is a linear increase in ΔpH as extracellular pH declines (4). Volatile fatty acids had little effect on *E. coli* when the pH was neutral, but there was a marked decrease in growth rate when the extracellular pH was <7.0 and the ΔpH was 0.75 (17). *Fibrobacter* (*Bacteroides*) *succinogenes* (11) and *Lactococcus cremoris* (9) also increased ΔpH as extracellular pH declined, and neither of the species can tolerate volatile fatty acids at low pH.

The observation that some bacteria let intracellular pH decrease introduces other questions. Why do some bacteria create a large ΔpH in the first place? Is intracellular metabolism really sensitive to a decline in intracellular pH per se? In *E. coli*, ΔpH is created at the expense of the electrical potential across the cell membrane (Δψ), but as Kaback (4) recently noted, "the mechanism responsible for the maintenance of internal pH at relatively acid pH values remains a fascinating, but unresolved problem."

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REFERENCES

1. **Baronofsky, J. J., W. J. A. Schreurs, and E. R. Kashket.** 1984. Uncoupling by acetic acid limits growth of and acetogenesis by *Clostridium thermoaceticum*. Appl. Environ. Microbiol. **48:** 1134–1139.
2. **Chen, G., and J. B. Russell.** 1991. More monensin-sensitive, ammonia-producing bacteria from the rumen. Appl. Environ. Microbiol. **55:**1052–1057.
3. **Hollowell, C. A., and M. J. Wolin.** 1965. Basis of the exclusion of *Escherichia coli* from the rumen ecosystem. Appl. Microbiol. **13:**918–924.
4. **Kaback, H. R.** 1990. Active transport: membrane vesicles, bioenergetics, molecules and mechanisms, p. 515–193. *In* T. A. Krulwich (ed.), Bacterial energetics. Academic Press, Inc., New York.
5. **Kashket, E. R.** 1985. The proton motive force in bacteria: a critical assessment of methods. Annu. Rev. Microbiol. **39:**219–242.
6. **Kashket, E. R.** 1987. Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. FEMS Microbiol. Rev. **46:**233–244.
7. **Luli, G. W., and W. R. Strohl.** 1990. Comparison of growth, acetate production, and acetate inhibition of *Escherichia coli* strains in batch and fed-batch fermentations. Appl. Environ. Microbiol. **56:**1004–1011.
8. **Padan, E., D. Zilberstein, and S. Schuldiner.** 1981. pH homeostasis in bacteria. Biochim. Biophys. Acta **650:**151–166.
9. **Poolman, B., A. J. M. Driessen, and W. N. Konings.** 1987. Regulation of solute transport in streptococci by external and internal pH values. Microbiol. Rev. **51:**498–508.
10. **Rogosa, M., J. A. Mitchell, and R. Wiseman.** 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol. **62:**132–133.
11. **Russell, J. B.** 1987. Effect of extracellular pH on growth and proton motive force of *Bacteroides succinogenes*, a cellulolytic ruminal bacterium. Appl. Environ. Microbiol. **53:**2379–2383.
12. **Russell, J. B.** 1991. Resistance of *Streptococcus bovis* to acetic acid at low pH: relationship between intracellular pH and anion accumulation. Appl. Environ. Microbiol. **57:**255–259.
13. **Russell, J. B., and D. B. Dombrowski.** 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Appl. Environ. Microbiol. **39:**604–610.
14. **Russell, J. B., and T. Hino.** 1985. Regulation of lactate production in *Streptococcus bovis*: a spiraling effect that leads to rumen acidosis. J. Dairy Sci. **68:**1712–1721.
15. **Salmond, C. V., R. H. Kroll, and I. R. Booth.** 1984. The effect of food preservatives on pH. J. Gen. Microbiol. **130:**2845–2950.
16. **Sheu, C. W., D. Salomon, T. Sheevalsan, and E. Freese.** 1975. Inhibitory effects of lipophilic acids and related compounds on bacteria and mammalian cells. Antimicrob. Agents Chemother. **7:**349–363.
17. **Wallace, R. J., M. L. Falconer, and P. K. Bhargava.** 1989. Toxicity of volatile fatty acids at rumen pH prevents enrichment of *Escherichia coli* by sorbitol in rumen contents. Curr. Microbiol. **19:**277–281.