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Effect of pH and Monensin on Glucose Transport by *Fibrobacter succinogenes*, a Cellulolytic Ruminal Bacterium

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***Fibrobacter succinogenes* S85, a cellulolytic ruminal bacterium, required sodium for growth and glucose uptake. Cells which were deenergized with iodoacetate (500 μM) could not take up [^{14}C]glucose. However, deenergized cells which were treated with valinomycin, loaded with potassium, and diluted into sodium or sodium plus potassium to create an artificial electrical gradient ($\Delta\psi$) plus a chemical gradient of sodium (ΔpNa) or ΔpNa alone transported glucose at a rapid rate. Cells which were loaded with potassium plus sodium and diluted into sodium ($\Delta\psi$ with sodium, but no ΔpNa) also took up glucose at a rapid rate. Potassium-loaded cells that were diluted into buffers which did not contain sodium ($\Delta\psi$ without sodium) could not take up glucose. An artificial ZΔpH which was created by acetate diffusion could not drive glucose transport even if sodium was present. The maximum rate and affinity of glucose transport (pH 6.7) were 62.5 nmol/mg of protein per min and 0.51 mM, respectively. S85 was unable to grow at a pH of less than 5.5, and there was little glucose transport at this pH. When the extracellular pH was decreased, the glucose carrier was inhibited, intracellular pH declined, the cells were no longer able to metabolize glucose, and $\Delta\psi$ declined. Monensin (1 μM) or lasalocid (5 μM) decreased intracellular ATP and dissipated both the $\Delta\psi$ and ΔpNa. Since there was no driving force for transport, glucose transport was inhibited. These results indicated that *F. succinogenes* used a pH-sensitive sodium symport mechanism to take up glucose and that either a $\Delta\psi$ or a ΔpNa was required for glucose transport.**

Since the mid 1970s, the ionophores monensin and lasalocid have been routinely fed to fattening beef cattle, which consume large amounts of cereal grain (28). The efficiency of feed utilization generally increases, and there is often a decline in food intake. More recently, ionophores have been approved for use in grazing animals, and the response has been even greater (11). The effect of ionophores on ruminal bacteria has not been studied in great detail, but gram-positive bacteria which lack an outer membrane to protect the cell membrane are usually more sensitive than gram-negative bacteria (27).

When *Streptococcus bovis*, an acid-tolerant, gram-positive ruminal bacterium, was treated with monensin, there was a large decrease in intracellular potassium, an influx of sodium and protons, and a decrease in ATP (22). The membrane potential ($\Delta\psi$) was unaffected, and this effect was consistent with the electroneutral action of this metal and proton antiporter (20). Although growth was inhibited, monensin had relatively little effect on the rate of glucose utilization (22, 29). In *S. bovis*, glucose is transported by the phosphotransferase system (19) or by facilitated diffusion (24).

Fibrobacter (*Bacteroides*) *succinogenes* was first isolated from the rumen by Hungate in 1947 (14), and subsequent work indicated that it was one of the most active degraders of crystalline cellulose (2, 12). Ruminal cellulolytic bacteria are sensitive to even modest declines in pH (26), and later work indicated that *F. succinogenes* could not maintain a membrane potential across the cell membrane at a low pH (23). Although *F. succinogenes* is a gram-negative bacterium, Chen and Wolin (8) noted that the S85 strain was initially sensitive to monensin.

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F. succinogenes has an absolute requirement for sodium (3), and recent work by Franklund and Glass (10) showed that glucose transport was stimulated by sodium and inhibited by monensin and lasalocid. While these latter results suggested that glucose transport was sodium dependent, there was little information regarding (i) the role of sodium in glucose transport, (ii) the driving force for glucose uptake, and (iii) the effect of pH and ionophores on glucose transport.

MATERIALS AND METHODS

Organism and growth conditions. *F. succinogenes* S85 was grown anaerobically in media containing (per liter) 292 mg of K_2HPO_4, 292 mg of KH_2PO_4, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4 g of Na_2CO_3, 1.0 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 0.5 g of yeast extract, 0.6 g of cysteine hydrochloride, 20 mmol of glucose, and a volatile fatty acid mixture (4). The cultures were incubated (39°C) in 500-ml vessels which were continuously purged with O_2-free CO_2 or in tubes (18 by 150 mm) which were sealed with butyl rubber stoppers. Growth was monitored by the increase in optical density (600 nm, 1-cm cuvette). Monensin or lasalocid was dissolved in ethanol, and the final concentration of ethanol was less than 2% (vol/vol).

Proton motive force. Exponentially growing cultures were anaerobically transferred with a hypodermic syringe (2.0 ml) to tubes (13 by 100 mm) which contained [U-^{14}C]tetraphenylphosphonium ion (TPP^+) (0.5 μCi, 30 μCi/μmol), [^{3}H]TPP^+ (0.5 μCi, 23 μCi/μmol), ^3H_2O (4 μCi), [U-^{14}C]sucrose (0.4 μCi, 540 μCi/mmol), or [7-^{14}C]benzoic acid (0.5 μCi, 22 μCi/μmol). After 5 min of incubation at 39°C, the cultures were centrifuged (13,000 × *g*, 5 min) through silicon oil (50:50 mixture of Hysol 550 and 560 [Hysol Co., Olean,

N.Y.], incubated overnight in an anaerobic glove box to remove O_2). Supernatant samples (20 μl) were removed, and the remaining sample was frozen (−15°C). The pellets at the bottoms of the tubes were removed with a pair of dog nail clippers. Supernatant samples and cell pellets were counted by liquid scintillation.

The intracellular volume (2.8 μl/mg of protein) was estimated from the relative uptake of ^3H_2O (4 μCi) and [U-^{14}C]sucrose (0.4 μCi, 540 μCi/mmol), a nonmetabolizable sugar. Δψ was calculated from the uptake of TPP^+ by using the Nernst equation (62 mV × log [in]/[out]), and the nonspecific binding of TPP^+ was estimated from cells which had been treated with nigericin plus valinomycin (10 μM each) or toluene (1% of a 1:9 [vol/vol] toluene to ethanol). The ZΔpH was estimated from the uptake of benzoate by using the Henderson-Hasselbalch equation (21), and ZΔpH was calculated as 62 mV × ΔpH.

Sodium and potassium determinations. Exponentially growing cultures (4 ml) were centrifuged through 0.3 ml of silicon oil as described above. The cell pellets and supernatant samples (10 μl) were digested at room temperature for 24 h in 3 N HNO_3, and insoluble cell debris was removed by centrifugation (33,000 × *g*, 15 min). Sodium and potassium were determined by flame photometry (Cole-Parmer 2655-00 Digital Flame Analyzer; Cole-Parmer Instrument Co., Chicago, Ill.). Cell pellets were corrected for extracellular contamination (internal to external volume, 1.33:1.00).

Transport by whole cells. Exponentially growing cultures were washed twice in anaerobic buffer (per liter: 292 mg of K_2HPO_4, 292 mg of KH_2PO_4, 240 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4 g of Na_2CO_3, and 0.6 g of cysteine hydrochloride, pH 6.7), incubated at 39°C with D-[U-^{14}C]glucose (0.08 μCi, 4.0 μCi/μmol) for 0 to 120 s, filtered through 0.65-μm-pore-size nitrocellulose membrane filters (Millipore), and washed with 4 ml of 0.1 M LiCl. Filters were dried for 20 min at 100°C, and the uptake of ^{14}C-labeled glucose was counted by liquid scintillation. In some cases, sodium salts were replaced by potassium salts (<0.1 mM sodium).

Artificial diffusion potentials. Exponentially growing cultures were deenergized with 500 μM iodoacetate (39°C for 30 min) and washed twice with 50 mM K_2HPO_4 plus 10 mM $MgSO_4 \cdot 7H_2O$ (pH 7.0) with or without 100 mM NaCl. The concentrated (100-fold) cell suspension was treated with 100 μM valinomycin (0°C, 30 min), and an artificial electrical gradient (Δψ) was generated by diluting potassium-loaded cells 50-fold into 100 mM Tris plus 10 mM $MgSO_4 \cdot 7H_2O$ (pH 7.0 unless otherwise stated). MES [2-(*N*-morpholino) ethanesulfonic acid] replaced Tris in the pH experiments. A chemical gradient of sodium (ΔpNa) was created by diluting the same cells into Tris plus 100 mM NaCl and KCl. Potassium-loaded cells were diluted into Tris containing 100 mM NaCl to create both a Δψ and a ΔpNa. A Δψ (with sodium, but no ΔpNa) was created by diluting sodium plus potassium-loaded cells into sodium. If potassium-loaded cells were diluted into 100 mM KCl, no force was generated. An artificial proton gradient in the presence of sodium was generated by loading valinomycin-treated cells in a solution of 100 mM NaCl, 80 mM potassium acetate, 10 mM K_2HPO_4, and 10 mM $MgSO_4 \cdot 7H_2O$ (pH 7.0) and diluting the cells into the same buffer which lacked acetate.

Binding studies. Ruminal contents were obtained from a ruminally fistulated nonlactating dairy cow, 1.5 h after feeding (2.5 kg of timothy hay twice daily). The contents were squeezed through cheesecloth and incubated at 39°C. Once the feed particles had been buoyed to the top of the flask by gas production, mixed ruminal bacteria were obtained from the center of the flask. The mixed ruminal bacterial suspension was then incubated with [^{14}C]lasalocid (6.4 nCi/ml, 1.74 μCi/mg) for 4 h at 22°C. The bacterial suspension was then centrifuged (13,000 × *g*, 5 min, 22°C), and the supernatant (0.5 ml) was counted by liquid scintillation.

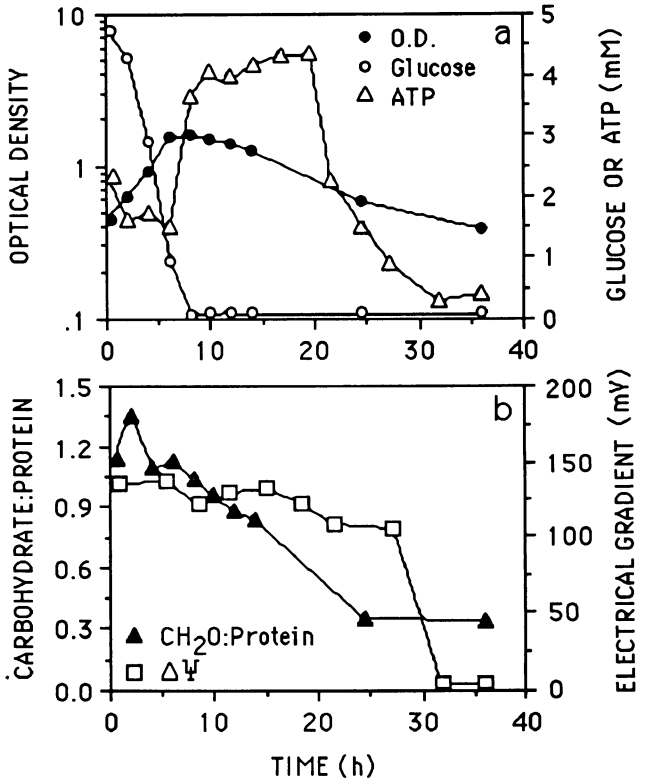

FIG. 1. Effects of glucose starvation on the optical density, intracellular ATP, carbohydrate-to-protein ratio, and membrane potential of *F. succinogenes* S85.

Other analyses. Glucose was assayed by an enzymatic procedure (1). Cell carbohydrate was measured by using Anthrone reagent, and cell protein was determined by the Lowry method (17). Samples for ATP determination were prepared as previously described (29) and assayed with a luminometer (model 1250; LKB Instruments, Inc., Gaithersburg, Md.) to measure light output of a luciferine-luciferase mix (Sigma Chemical Co., St. Louis, Mo.).

RESULTS

Cell growth. *F. succinogenes* S85 had a growth rate of 0.24 h^{-1} when glucose was provided as an energy source, and the cultures stopped growing when the external glucose was exhausted (Fig. 1a). After the glucose was depleted, there was a gradual decline (0.06 h^{-1}) in optical density, but there was an increase rather than a decrease in intracellular ATP. Exponentially growing cells had large amounts of polysaccharide, and intracellular ATP concentrations and the membrane potential (Δψ) did not decline until there was a substantial reduction in cellular polysaccharide (Fig. 1b).

S85 grew less rapidly at a low pH, and there was a decrease in ATP at low pH values (Fig. 2a). Growth ceased at pH 5.4, and the ATP concentration was less than 1 mM.

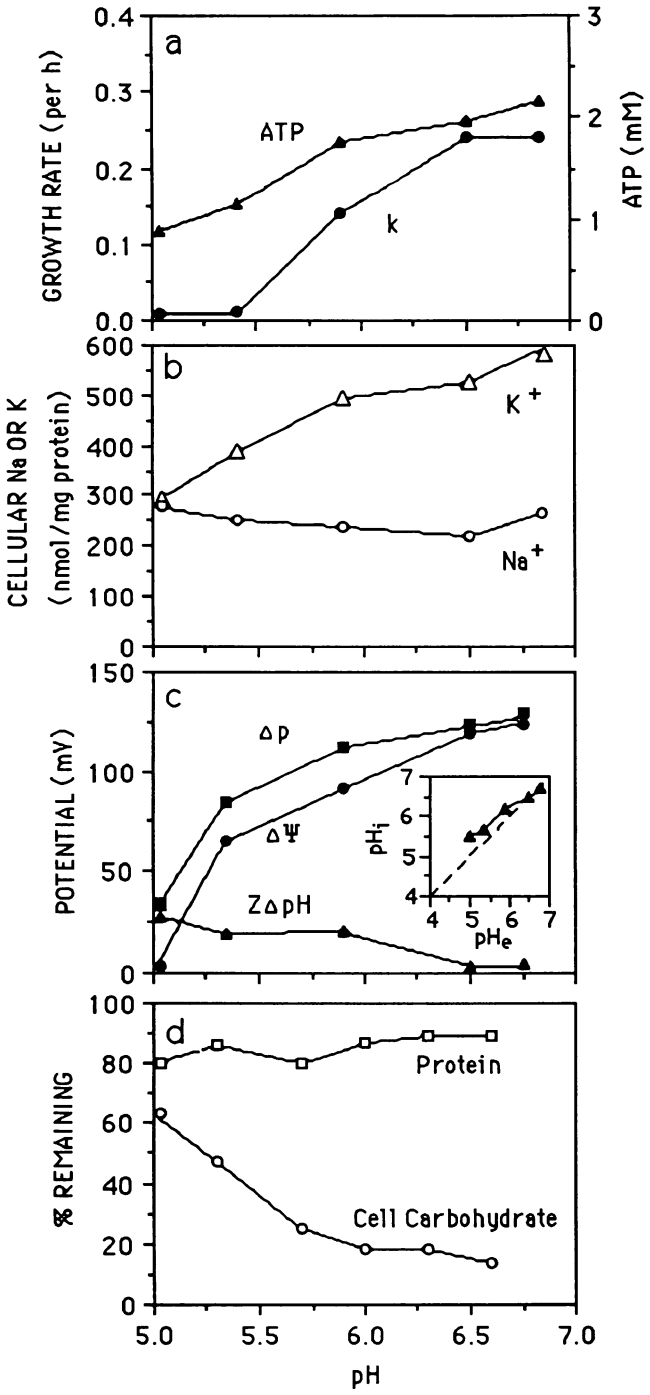

FIG. 2. Effects of pH on the growth rate and intracellular ATP concentration (a), cellular Na and K concentrations (b), and proton motive force of *F. succinogenes* S85 (c) during exponential growth. (d) Percentage of protein and cell carbohydrate that remained 10 h after external glucose was depleted. Extracellular pH was decreased by the addition of concentrated HCl.

As extracellular pH declined, there was a decline in cellular potassium, but pH had little effect on cellular sodium (Fig. 2b). At a low pH, ΔpH across the cell membrane increased, but there was a large decrease in intracellular pH, Δψ, and Δp (Fig. 2c). Stationary cultures which were incubated at a

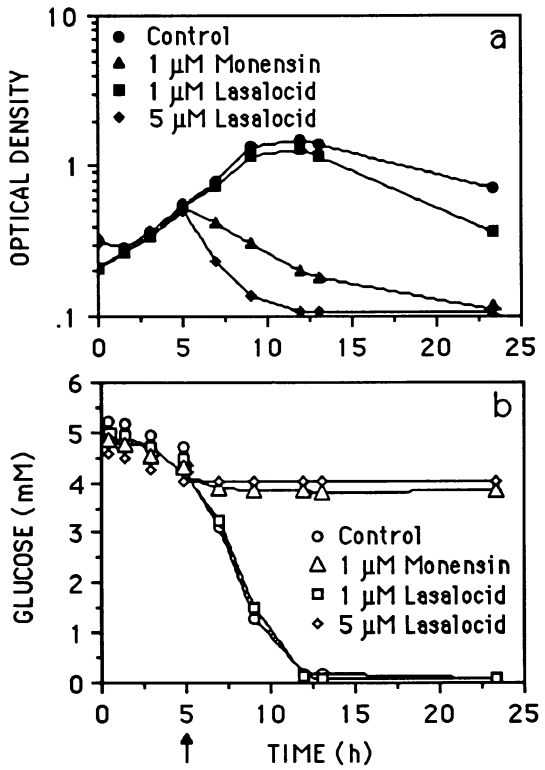

FIG. 3. Growth (a) and glucose utilization (b) by *F. succinogenes* S85. Cultures were treated with either monensin or lasalocid (arrow).

low pH for 10 h did not show a greater reduction in protein than cells which were incubated at near neutral pH, but the polysaccharide content was higher (Fig. 2d).

Exponentially growing cultures were inhibited by 1 μM monensin and 5 μM lasalocid, and there was a decline in optical density after the ionophore was added (Fig. 3a). When 500 mM sucrose was added to the medium as an osmotic stabilizer, there was a slower decline in optical density (data not shown). Both of the ionophores inhibited glucose utilization (Fig. 3b). Neither ionophore affected ZΔpH, but both ionophores were able to decrease intracellular ATP and dissipate the electrical, sodium, and potassium gradients across the cell membrane (Table 1). Iodoacetate (100 mM) also caused a decrease in membrane potential (data not shown).

Glucose transport. Cells which were washed and incubated in anaerobic buffer (pH 6.7) transported [^{14}C]glucose at a rate of 10 nmol/mg of protein per min, and uptake was linear for 60 s (Fig. 4). If the same cells were washed in sodium-deficient buffer, deenergized with iodoacetate (500 μM, 30 min), or washed aerobically, little uptake was observed. Deenergized cells which were treated with valinomycin, loaded with potassium, and diluted 50-fold into Tris buffer containing NaCl or LiCl (Δψ plus ΔpNa or ΔpLi) transported glucose at a rate of 4.1 nmol/mg of protein per min (Fig. 5), and oxygen had little, if any, effect on glucose transport. If the cells were loaded with both potassium and sodium and diluted into Tris buffer plus NaCl (Δψ with Na, but no ΔpNa), the initial rate of glucose transport was unaffected, but there was less [^{14}C]glucose accumulation. Cells which were loaded with potassium and diluted into potassium plus

TABLE 1. Effects of ionophores on proton motive force and ion gradients of *F. succinogenes* S85

Parameter (unit)	Value[a] for the following culture:				
	Control	Monensin		Lasalocid	
		1 μM	5 μM	1 μM	5 μM
Δψ (mV)	150	0	0	120	0
pH_{ex}	6.7	6.7	6.7	6.7	6.7
pH_{in}	6.6	6.7	6.7	6.7	6.7
ZΔpH	0	0	0	0	0
Δp (mV)	140	0	0	120	0
Na_{ex} (mM)	130	130	120	130	120
Na_{in} (μmol/mg of protein)	0.3	1.1	1.9	0.3	2.2
K_{ex} (mM)	5	5	3	5	4
K_{in} (μmol/mg of protein)	0.5	0.1	0.1	0.5	0.2
ATP (mM)	3.1	ND	0.8	ND	1.0

[a] ND, not determined.

sodium (ΔpNa, but no Δψ) took up glucose at a slower rate (1 nmol/mg of protein per min).

Deenergized and valinomycin-treated cells which were diluted into Tris buffer lacking NaCl (Δψ, but no sodium) or Tris buffer plus 100 mM KCl (no driving force) did not take up glucose. Cells which were loaded with acetate and diluted (50-fold) into Tris buffer to create a chemical gradient of protons (ZΔpH) were also unable to transport glucose even if 100 mM NaCl was added to the loading and dilution buffers.

When Δψ plus ΔpNa was used as the driving force, there was a linear decline in the rate of glucose transport as the pH was decreased from pH 7.0 to 5.5 (Fig. 6). No transport was observed at pH 5.5 or lower. When sodium was omitted from the transport buffer, little if any [^{14}C]glucose was transported, but rapid uptake was observed if as little as 5 mM NaCl was added (Fig. 7). If more than 80 mM NaCl was added, there was a small but significant reduction in glucose transport. An Eadie-Hofstee plot of glucose transport indicated that the affinity constant (K_t) was 0.51 mM with a maximum rate (V_{max}) of 62.5 nmol/mg of protein per min (data not shown).

Ionophore binding. When mixed ruminal bacteria were incubated in buffer containing 6 μM ^{14}C-labeled lasalocid, there was a 40% reduction in the amount of ionophore remaining in suspension.

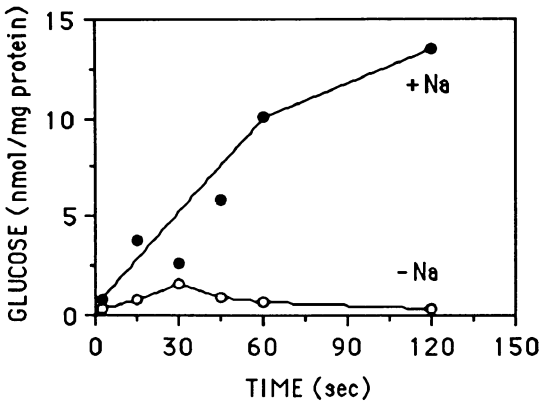

FIG. 4. Glucose transport by *F. succinogenes* S85 cells which were washed and incubated in anaerobic media (pH 6.7) which contained or lacked sodium.

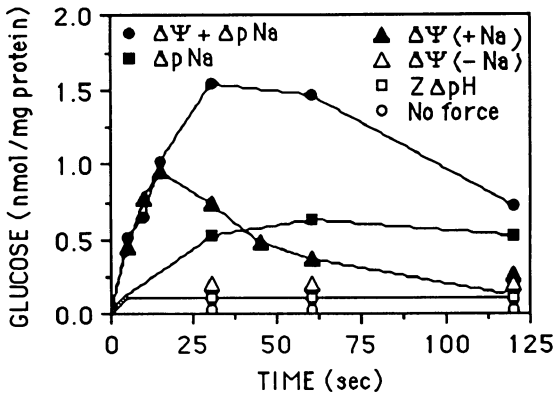

FIG. 5. Glucose transport by *F. succinogenes* S85 cells which were treated with iodoacatate (500 μM) and valinomycin (100 μM). An artificial diffusion potential (Δψ) was created by potassium diffusion, and a ZΔpH was created by acetate diffusion.

DISCUSSION

Gram-negative ruminal bacteria are generally resistant to ionophores (28), but Chen and Wolin noted that unadapted cultures of *F. succinogenes* S85, a gram-negative bacterium, could not grow in medium which contained 3.6 μM monensin or lasalocid (8). Their adapted cultures eventually tolerated 28 and 14 μM monensin and lasalocid, respectively, but other workers were unable to achieve as great a degree of resistance (<8 μM [13]). We were never able to grow the S85 strain with 1 μM monensin, and it could tolerate only 1 μM lasalocid.

Because gram-positive ruminococci are the only other active group of cellulolytic bacteria in the rumen (8), it has been assumed that *F. succinogenes* would occupy the cellulose-digesting niche of animals fed ionophores (13). On the basis of a recommended daily dose of 350 mg of monensin per day and a rumen volume of 70 liters, the in vivo concentration would be approximately 7.2 μM. On the basis of this calculation, it seems unlikely that *F. succinogenes* would persist in vivo, but this calculation makes no allowance for the higher numbers of bacteria in vivo. Preliminary experiments indicated that ruminal bacteria could bind large amounts of ionophore so that the in vivo concentration could

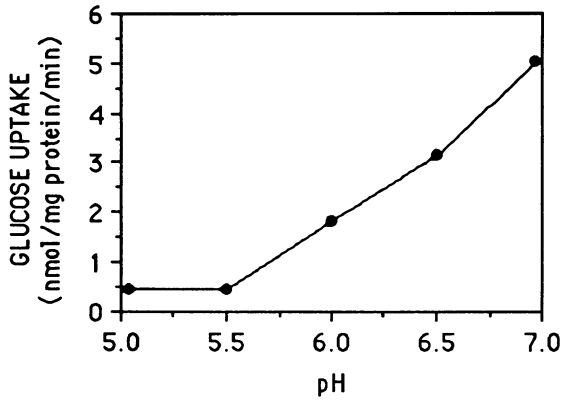

FIG. 6. Effects of pH on glucose transport by *F. succinogenes* S85. Iodoacetate- and valinomycin-treated cells were loaded with potassium buffer (pH 5.0 to 7.0) and diluted into sodium MES (pH 5.0 to 7.0). Transport was measured over a 30-s period.

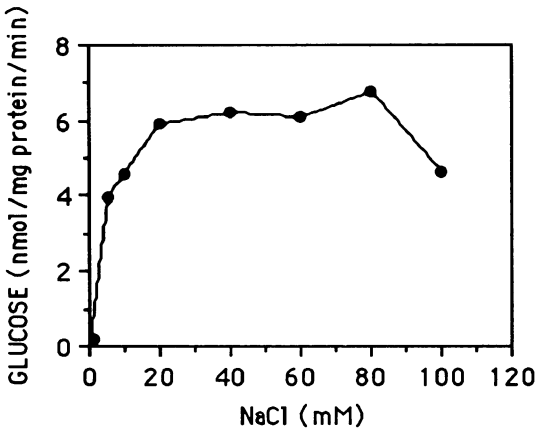

FIG. 7. Effects of added sodium on the rate of glucose transport by deenergized and valinomycin-treated *F. succinogenes*. Cells were incubated in potassium phosphate and diluted into Tris (pH 7.0).

be significantly lower. When 6 μM ^{14}C-labeled lasalocid was added to rumen liquor, the bacteria adsorbed more than 40% of the ionophore. Since even more ruminal bacteria are associated with the particulate (plant) fraction, it is likely that free ionophore concentrations in vivo are significantly less than 7.2 μM.

The rumen is a sodium-rich environment which has been likened to an "inland sea" (5). Many species of rumen bacteria require sodium for growth (5, 32), but there is little information regarding the role of sodium in bacteria (16, 18). Sodium can be involved in a variety of membrane-related functions (transport, intracellular pH regulation, osmoregulation, etc.), and recent studies have shown that several species of rumen bacteria had sodium cotransport systems (6, 7, 30, 32).

Franklund and Glass (10) indicated that sodium stimulated glucose uptake by *F. succinogenes*, but an absolute requirement for sodium was never demonstrated. Our experiments showed that (i) sodium was always required for glucose transport, (ii) a $\Delta\psi$, a ΔpNa, or both a $\Delta\psi$ and a ΔpNa could drive transport, and (iii) a ZΔpH could not drive transport even in the presence of sodium. On the basis of these results, it appeared that *F. succinogenes* took up glucose by a sodium symport mechanism. Many sodium-dependent carriers can use lithium (18), and the glucose transport system of *F. succinogenes* can also use a lithium gradient.

The *F. succinogenes* glucose carrier had a much lower affinity for sodium than some *Escherichia coli* sodium cotransport systems (e.g., K_t of 4 versus 21 mM [18]), but the rumen has approximately 100 mM sodium. Maximal rates of glucose transport were observed when the sodium concentration was 80 mM, but there was some decrease in the rate of transport when the sodium concentration was increased to 100 mM. It is not clear why high salt concentrations inhibit sodium symport, but Maloy (18) suggested that sodium could have nonspecific effects on the membrane structure or cause specific conformational changes in the transporter.

In the rumen, sugar concentrations are usually low, and substrate affinity can be a key factor influencing bacterial competition (25). *F. succinogenes* had a K_t for glucose of 510 μM, but *Butyrivibrio fibrisolvens* and *Selenomonas ruminantium* had K_s values of 9 and 46 μM (25). Since *F. succinogenes* is a cellulolytic bacterium that can attach firmly to cellulose (12), a high affinity for sugars may not be necessary, and cellulose digestion may yield cellobiose and cellodextrins rather than glucose.

The rumen is well buffered by sodium carbonate, but pH can decline if the rate of carbohydrate fermentation is rapid (31). *F. succinogenes* was unable to utilize glucose or grow at a low pH (Fig. 2a). At a low pH, $\Delta\psi$ declined (Fig. 2c), but glucose transport was also inhibited even if a $\Delta\psi$ plus ΔpNa was used as the driving force (Fig. 6). On the basis of this result, it appeared that pH was having a direct effect on the glucose carrier. When extracellular pH declined, *F. succinogenes* attempted to increase its ΔpH, but there was eventually a reduction in intracellular pH (Fig. 2c). Because the cells could not utilize polysaccharide as an energy source for endogenous metabolism at low pH (Fig. 2d), it appeared that low intracellular pH was inhibiting metabolism. When carbohydrate metabolism was inhibited, ATP and $\Delta\psi$ declined.

Previous work showed that monensin inhibited the growth of *S. bovis*, but glucose fermentation continued (22). *S. bovis* uses the phosphotransferase system (19) and facilitated diffusion (24) to transport glucose, and neither of these mechanisms depends upon ion gradients across the cell membrane. *F. succinogenes* does not have a glucose phosphotransferase system, and monensin and lasalocid strongly inhibited glucose transport. The inhibition of glucose transport was most easily explained by the collapse of $\Delta\psi$ and ΔpNa (Table 1).

Monensin and lasalocid are metal and proton antiporters, and in *S. bovis*, monensin had little effect on $\Delta\psi$ (22). When either 1 μM monensin or 5 μM lasalocid was added to *F. succinogenes*, there was a large reduction in $\Delta\psi$, and this decrease was inconsistent with the electroneutral action of the compounds (Table 1). However, it should be noted that both of these compounds caused a large reduction in ATP. When *F. succinogenes* was treated with iodoacetate, an inhibitor of glycolysis, there was also a reduction in ATP and $\Delta\psi$. On the basis of these observations, it appeared that the efflux of potassium and influx of sodium caused a reduction in ATP and that the reduction in ATP in turn led to a decrease in $\Delta\psi$.

S. bovis did not lyse when it was treated with monensin (9, 22), but there was an immediate decrease in optical density when ionophores were added to *F. succinogenes* (Fig. 3). *Bacillus subtilis* lyses whenever there is a decrease in $\Delta\psi$ or ZΔpH, and Jolliffe et al. (15) suggested that autolysin activity was regulated by membrane energization. However, *F. succinogenes* did not lyse at a low pH even though $\Delta\psi$ was dissipated. Since monensin-induced lysis was prevented by 500 mM sucrose, it appeared that lysis was caused by an increase in the internal osmotic pressure. Iodoacetate (100 μM) and tetrachlorosalicylanilide (0.5 μM) also caused a rapid decrease in optical density (data not shown).

Monensin and lasalocid had similar effects on ion flux (Table 1), but their potencies can differ. Low concentrations of monensin (1 μM) were much more effective against *F. succinogenes* than lasalocid, and this difference may be related to the hydrophobicity of the ionophores. Since lasalocid is more lipophilic than monensin, more lasalocid may be trapped in the outer membrane and less reaches the cell membrane. Lasalocid was more inhibitory to *S. bovis* than monensin (9), but this bacterium lacks an outer membrane to protect the cell membrane.

When bacteria exhaust their energy supply, there is usually an immediate decline in ATP (29). *F. succinogenes*

stopped growing as soon as the glucose was depleted, but there was an increase, rather than a decrease, in intracellular ATP. Polysaccharide utilization supplied energy to sustain ATP levels and the $\Delta\psi$, but the question of why the cells stopped growing remains. This observation indicates that some factor other than ATP supply must regulate growth.

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REFERENCES

1. **Bergemeyer, H. U., and H. Klotsch.** 1965. Sucrose, p. 99–102. *In* H. U. Bergemeyer (ed.), Methods of enzymatic analysis. Academic Press, Inc., New York.
2. **Bryant, M. P.** 1954. A study of actively cellulolytic rod-shaped bacteria of the bovine rumen. J. Dairy Sci. **37:**1176–1183.
3. **Bryant, M. P., I. M. Robinson, and H. Chu.** 1959. Observations on the nutrition of *Bacteroides succinogenes*—a ruminal cellulolytic bacterium. J. Dairy Sci. **42:**1831–1847.
4. **Caldwell, D. R., and M. P. Bryant.** 1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. Appl. Microbiol. **14:**794–801.
5. **Caldwell, D. R., and R. F. Hudson.** 1974. Sodium, an obligate growth requirement for predominant rumen bacteria. Appl. Microbiol. **27:**539–552.
6. **Chen, G., and J. B. Russell.** 1989. Sodium-dependent transport of branched-chain amino acids by a monensin-sensitive ruminal peptostreptococcus. Appl. Environ. Microbiol. **55:**2658–2663.
7. **Chen, G., and J. B. Russell.** 1990. Transport and deamination of amino acids by a gram-positive, monensin-sensitive ruminal bacterium. Appl. Environ. Microbiol. **56:**2186–2192.
8. **Chen, M., and M. J. Wolin.** 1979. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. Appl. Environ. Microbiol. **38:**72–77.
9. **Chow, J. M., and J. B. Russell.** 1990. Effect of ionophores and pH on growth of *Streptococcus bovis* in batch and continuous culture. Appl. Environ. Microbiol. **56:**1588–1593.
10. **Franklund, C. V., and T. L. Glass.** 1987. Glucose uptake by the cellulolytic ruminal anaerobe *Bacteroides succinogenes*. J. Bacteriol. **169:**500–506.
11. **Goodrich, R. D., J. E. Garrett, D. R. Gast, M. A. Kirick, D. A. Larson, and J. C. Meiske.** 1984. Influence of monensin on the performance of cattle. J. Anim. Sci. **58:**1484–1498.
12. **Halliwell, G., and M. P. Bryant.** 1963. The cellulolytic activity of pure strains of bacteria from the rumen of cattle. J. Gen. Microbiol. **32:**441–448.
13. **Henderson, C., C. S. Stewart, and F. V. Nekrep.** 1981. The effect of monensin on pure and mixed cultures of rumen bacteria. J. Appl. Bacteriol. **51:**159–169.
14. **Hungate, R. E.** 1947. Studies on cellulose fermentation. III. The culture and isolation of cellulose-decomposing bacteria from the rumen of cattle. J. Bacteriol. **53:**631–645.
15. **Joliffe, L. K., E. J. Doyle, and U. N. Streips.** 1981. The energized membrane and cellular autolysis in *Bacillus subtilis*. Cell **25:**753–763.
16. **Lanyi, J. K.** 1979. The role of Na^+ in transport processes of bacterial membranes. Biochim. Biophys. Acta **559:**377–397.
17. **Lowry, O. H., N. J. Rosehough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
18. **Maloy, S. R.** 1990. Sodium-coupled cotransport, p. 203–224. *In* T. A. Krulwich (ed.), The bacteria, vol. 12. Bacterial energetics. Academic Press, Inc., New York.
19. **Martin, S. A., and J. B. Russell.** 1987. Transport and phosphorylation of disaccharides by the ruminal bacterium *Streptococcus bovis*. Appl. Environ. Microbiol. **53:**2388–2393.
20. **Pressman, B. C.** 1976. Biological applications of ionophores. Annu. Rev. Biochem. **45:**501–530.
21. **Reibeling, V., R. K. Thauer, and K. Jungermann.** 1975. The internal-alkaline pH gradient, sensitive to uncoupler and ATPase inhibitor, in growing *Clostridium pasteurianum*. Eur. J. Biochem. **55:**445–453.
22. **Russell, J. B.** 1987. A proposed model of monensin action in inhibiting rumen bacterial growth: effects on ion flux and protonmotive force. J. Anim. Sci. **64:**1519–1525.
23. **Russell, J. B.** 1987. Effect of extracellular pH on growth and proton motive force of *Fibrobacter succinogenes*, a cellulolytic ruminal bacterium. Appl. Environ. Microbiol. **53:**2379–2383.
24. **Russell, J. B.** 1990. Low-affinity, high-capacity system of glucose transport in the ruminal bacterium *Streptococcus bovis*: evidence for a mechanism of facilitated diffusion. Appl. Environ. Microbiol. **56:**3304–3307.
25. **Russell, J. B., and R. L. Baldwin.** 1979. Comparison of substrate affinities among several rumen bacteria: a possible determinant of rumen bacterial competition. Appl. Environ. Microbiol. **37:**531–536.
26. **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.
27. **Russell, J. B., and H. J. Strobel.** 1988. Effects of additives on in vitro ruminal fermentation: a comparison of monensin and bacitracin, another gram-positive antibiotic. J. Anim. Sci. **66:**552–558.
28. **Russell, J. B., and H. J. Strobel.** 1989. Effects of ionophores on ruminal fermentation. Appl. Environ. Microbiol. **55:**1–6.
29. **Russell, J. B., and H. J. Strobel.** 1990. ATPase-dependent energy spilling by the ruminal bacterium, *Streptococcus bovis*. Arch. Microbiol. **153:**378–383.
30. **Russell, J. B., H. J. Strobel, A. J. M. Driessen, and W. N. Konings.** 1988. Sodium-dependent transport of neutral amino acids by whole cells and membrane vesicles of *Streptococcus bovis*, a ruminal bacterium. J. Bacteriol. **170:**3531–3536.
31. **Slyter, L. L.** 1976. Influence of acidosis on rumen function. J. Anim. Sci. **43:**910–929.
32. **Strobel, H. J., and J. B. Russell.** 1991. Role of sodium in the growth of a ruminal selenomonad. Appl. Environ. Microbiol. **57:**1663–1668.