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Succinate Transport by a Ruminal Selenomonad and Its Regulation by Carbohydrate Availability and Osmotic Strength

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Washed cells of strain H18, a newly isolated ruminal selenomonad, decarboxylated succinate 25-fold faster than *Selenomonas ruminantium* HD4 (130 versus 5 nmol min^{-1} mg of $protein^{-1}$, respectively). Batch cultures of strain H18 which were fermenting glucose did not utilize succinate, and glucose-limited continuous cultures were only able to decarboxylate significant amounts of succinate at slow (<0.1 h^{-1}) dilution rates. Strain H18 grew more slowly on lactate than glucose (0.2 versus 0.4 h^{-1}, respectively), and more than half of the lactate was initially converted to succinate. Succinate was only utilized after growth on lactate had ceased. Although nonenergized and glucose-energized cells had similar proton motive forces and ATP levels, glucose-energized cells were unable to transport succinate. Transport by nonenergized cells was decreased by small increases in osmotic strength, and it is possible that energy-dependent inhibition of succinate transport was related to changes in cell turgor. Since cells which were deenergized with 2-deoxyglucose or iodoacetate did not transport succinate, it appeared that glycogen metabolism was providing the driving force for succinate uptake. An artificial ΔpH drove succinate transport in deenergized cells, but an artificial membrane potential (Δψ) could not serve as a driving force. Because succinate is nearly fully dissociated at pH 7.0 and the transport process was electroneutral, it appeared that succinate was taken up in symport with two protons. An Eadie-Hofstee plot indicated that the rate of uptake was unusually rapid at high substrate concentrations, but the low-velocity, high-affinity component could account for succinate utilization by stationary cultures. The high-velocity, low-affinity system could play a role in succinate efflux. There was no evidence that succinate transport by either strain H18 or strain HD4 was electrogenic or stimulated by sodium.

Succinate is produced by a variety of ruminal bacteria (e.g., ruminococci, bacteroides, and succinivibrio), but little if any succinate ever accumulates in the rumen (11). Sijpesteijn and Elsden (30) noted that mixed ruminal bacteria decarboxylated succinate, and Blackburn and Hungate estimated that succinate decarboxylation accounted for approximately one-third of the ruminal propionate (3). Ruminal propionibacteria (12) and a *Veillonella* sp. (13) decarboxylated succinate, but these bacteria did not have sufficient activities or numbers to explain in vivo turnover rates.

Scheifinger and Wolin demonstrated that resting cells of *Selenomonas ruminantium* HD4 decarboxylated succinate and estimated that a cell density of 1.2×10^9/ml could account for succinate disappearance in vivo (29). When *S. ruminantium* was cocultured with *Bacteroides succinogenes*, little succinate accumulated and the succinate was stoichiometrically converted to propionate (29). On the basis of these results, it has generally been assumed that *S. ruminantium* is primarily responsible for utilization and turnover of ruminal succinate.

Although *S. ruminantium* is usually described as a succinate utilizer, the HD4 strain can also produce succinate (22). Since there was little information concerning the regulation of succinate utilization and production, we decided to examine the effects of carbohydrate availability on succinate uptake. Results indicated that succinate transport was strongly inhibited by glucose and that even small increases in osmotic strength decreased uptake.

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MATERIALS AND METHODS

Enrichment and isolation. Ruminal contents were obtained from a 600 kg, nonlactating dairy cow fed 2.5 kg of timothy hay and 2.5 kg of a commercial concentrate supplement. Ruminal digesta were collected 1.5 h after feeding and strained through eight layers of cheesecloth. Mixed ruminal bacteria (2-ml portions) were inoculated into duplicate tubes (8 ml) containing (per liter) 292 mg of K_2HPO_4, 292 mg of KH_2PO_4, 480 mg of Na_2SO_4, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot H_2O$, 600 mg of cysteine, 4 g of Na_2CO_3, 1 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 500 mg of yeast extract, 4 mg of biotin, and volatile fatty acids (4). The medium was adjusted to pH 6.7, and dulcitol and succinate were added as separate solutions (22 and 20 mM, respectively). All cultures were incubated at 39°C and transferred (20%, vol/vol) into fresh media every other day. After 1 week, the enrichments contained a predominance of crescent-shaped organisms (selenomonads).

After 40 days, cultures were streaked on agar plates (1% agar) containing the basal medium, dulcitol, and succinate. Strain H18 was obtained from a small (<2 mm), circular, off-white, slightly raised colony. The crescent-shaped cells were gram negative, had lateral flagella on one side, and required valeric acid. Lactate was the major product of glucose fermentation, and the isolate was able to convert lactate to propionate and acetate. The organism was cultivated on the basal medium described above, except that the volatile fatty acid solution was replaced by 1 mM valerate. When lactate was the energy source, 10 mM aspartate was also added.

Transport assays. Stationary cultures (11 mM glucose; 10 ml) were harvested by centrifugation (2,000 × *g*, 22°C, 5

min), washed, and resuspended with 200 μl of anaerobic buffer (50 mM K_2HPO_4, 10 mM $MgCl_2$ [pH 7.0]). Transport assays were conducted under nitrogen (200 μl of buffer with approximately 30 to 40 μg of protein). Uptake was initiated by the addition of [2,3-^{14}C]succinate (100 nCi; 110 mCi/mmol, final concentration of 4.55 μM). Preliminary experiments indicated that the velocity of transport was linear for 20 s. In some experiments, cells were washed and assayed in a solution containing 50 mM morpholinoethanesulfonic acid (MES), 50 mM morpholinopropanesulfonic acid (MOPS), and 10 mM $MgCl_2$ adjusted to pH 7.0 with choline base. Transport was terminated by the addition of 2 ml of ice-cold 100 mM lithium chloride and then filtration of the culture through a 0.45-μm-pore-size nitrocellulose membrane filter. Filters were washed with 2 ml of LiCl and then dried (105°C, 20 min), and radioactivity was determined by liquid scintillation.

Proton motive force determinations. An acid distribution method was used to determine intracellular pH (26). Cells (0.1 to 0.2 mg of protein per ml) were incubated (5 min, 39°C) with [7-^{14}C]benzoate (500 nCi; 21.8 mCi/mmol), [U-^{14}C] taurine (500 nCi; 115 mCi/mmol), or ^3H_2O (4,000 nCi) and centrifuged through silicon oil (50:50 mixture of Dow Corning 550 and 556; William F. Nye, Inc., New Bedford, Mass.). Supernatant samples (20 μl) were removed, and the bottoms of tubes containing cell pellets were removed with dog nail clippers after being frozen. Supernatants and pellets were mixed with scintillation cocktail, and radioactivity was determined by liquid scintillation. Intracellular volume (approximately 4 μl/mg of protein) was estimated by the difference in specific activities of [^{14}C]taurine and ^3H_2O. Membrane potential ($\Delta\psi$) was estimated from the uptake of [U-^{14}C] tetraphenylphosphonium bromide (500 nCi; 30 mCi/mmol; 5 min). Nonspecific binding of tetraphenylphosphonium bromide was estimated from cells treated with 1% toluene.

Artificial potentials. Stationary cultures (10 ml, approximately 200 mg of protein per liter) were deenergized with 2-deoxyglucose (2-DG; 11 mM, 30 min, 39°C) and washed in 50 mM potassium phosphate containing 10 mM $MgCl_2$ (pH 7.0). Deenergized cells were loaded with 50 mM acetate or treated with valinomycin (10 μM, 45 min, 0°C) to load potassium. Loaded cells were washed in potassium phosphate-acetate or potassium phosphate buffer and diluted into buffers containing 100 nCi of [^{14}C]succinate.

Intracellular metabolites. Stationary cultures grown on glucose were anaerobically harvested (400 mg of protein per liter), washed once with potassium phosphate buffer, and resuspended in 40 ml of buffer. Cells were then incubated for 5 min at 39°C with and without 20 mM glucose under nitrogen. The cell pellets (10,000 × *g*, 3 min, 0°C) were weighed and diluted with an equal volume of ice-cold 20% perchloric acid. After 4 h at 0°C the cell debris was removed by centrifugation. The acidified extract was neutralized with 1/2 volume of 2.5 M K_2CO_3, and insoluble salt was removed by centrifugation. Neutralized samples were then enzymatically assayed for glucose-6-phosphate (9), fructose-6-phosphate (9), and fructose-1,6-bisophosphate (25). Succinate was methylated (31) and determined by gas-liquid chromatography.

ATP determinations. Cells (0.1 to 0.2 mg of protein in 1 ml) were extracted with 0.5 ml of 14% perchloric acid (containing 9 mM EDTA, 20 min, 0°C), and insoluble cell debris was removed by centrifugation (5 min, 13,000 × *g*, 20°C). The supernatant (1 ml) was neutralized with 0.5 ml of KOH-K_2CO_3 (1 M each, 0°C). Neutralized extracts were diluted 50-fold with 40 mM Tris containing 2 mM EDTA, 10 mM $MgCl_2$, and 0.1% bovine serum albumin (pH 7.75), and 100 μl of diluted extract was mixed with 100 μl of purified luciferine-luciferase according to the recommendations of the manufacturer (Sigma Chemical Co., St. Louis, Mo.). Light output was measured with a luminometer (model 1250; LKB Instruments, Inc., Gaithersburg, Md.).

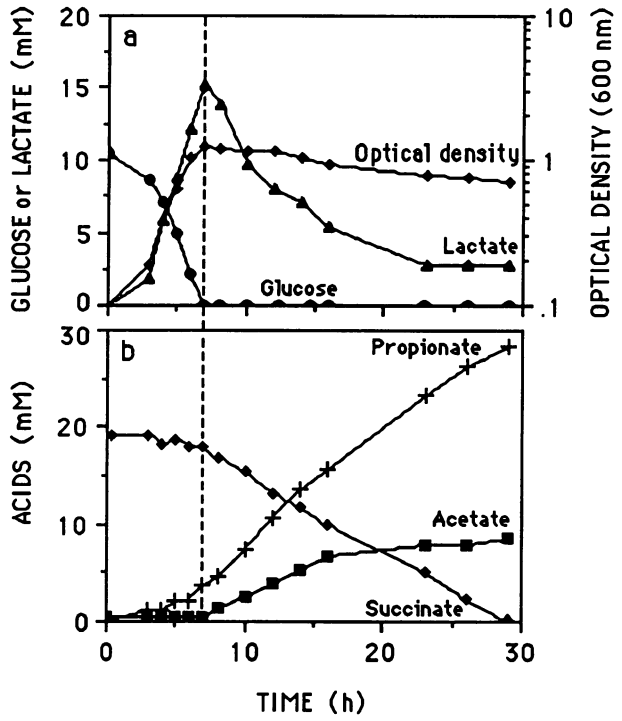

FIG. 1. Succinate and glucose utilization by glucose-grown strain H18.

Analyses. Succinate and volatile fatty acids in culture fluid were measured by high-pressure liquid chromatography with a Beckman model 334 liquid chromatograph and a Bio-Rad HPX-87H organic acid column (0.13 N H_2SO_4, 0.5 ml/min, 50°C, 20-μl sample). *meta*-Phosphoric acid-treated samples (4%, wt/vol) were also analyzed for volatile fatty acids by gas-liquid chromatography (Gow-Mac model 580 flame ionization gas chromatograph with Supelco 1220 column). Glucose and lactate were measured by enzymatic methods using hexokinase and glucose-6-phosphate dehydrogenase (1) and lactate dehydrogenase (10), respectively. Protein was determined by the method of Lowry et al. (19) after cells had been hydrolyzed (0.2 N NaOH, 15 min, 100°C).

Materials. All radiolabeled chemicals were obtained from Amersham, Arlington Heights, Ill. Tetrachlorosalianilide was purchased from Kodak, Rochester, N.Y., and all other chemicals were from commercial sources.

RESULTS

Succinate utilization. *S. ruminantium* HD4 decarboxylated succinate at a much slower rate than cultures of mixed ruminal bacteria (5 versus 100 nmol min^{-1} mg of $protein^{-1}$, respectively), but strain H18, a selenomonad which was isolated from the rumen with a dulcitol-succinate enrichment, decarboxylated succinate 25-fold faster than strain HD4 (130 versus 5 nmol min^{-1} mg of $protein^{-1}$, respectively). Stationary-phase cultures of strain H18 decarboxylated

succinate at a rapid rate, but batch cultures of strain H18 which were fermenting glucose did not utilize succinate (Fig. 1a). Glucose was initially converted to lactate, and no growth was observed after glucose depletion. Once growth had ceased, lactate was taken up and propionate and acetate were produced (Fig. 1b). Glucose depletion and lactate fermentation were associated with the onset of succinate metabolism, and preliminary experiments (data not shown) indicated that succinate was stoichiometrically decarboxylated to propionate.

Glucose-depleted cultures were unable to grow on lactate, but cultures that were initially provided with 20 mM lactate and 10 mM aspartate grew at a rate of 0.2 h^{-1} (Fig. 2a). During this time more than half of the lactate was converted to succinate. Although lactate was still available, growth eventually stopped, and the cessation of growth was associated with the onset of succinate utilization. Cultures provided with both lactate and succinate also produced a small amount of succinate early on and only used succinate after growth had ceased (Fig. 2b).

When strain H18 was grown in a glucose-limited chemostat supplemented with succinate, utilization was inversely related to dilution rate (Fig. 3a). As dilution rate was increased from 0.03 to 0.10 h^{-1}, there was an 80% reduction in succinate utilization. Since there was little change in microbial protein over these dilution rates, this decrease in succinate utilization could not be explained by washout. On the basis of the succinate utilization rate of stationary cultures, all of the succinate should have been utilized at dilution rates of less than 0.12 h^{-1}. When the pH of a slow-dilution-rate chemostat (0.05 h^{-1}) was decreased from 6.8 to 4.85, there was only a 25% reduction in succinate utilization (Fig. 3b). At pH 4.7, succinate was not utilized and this observation was explained by culture washout.

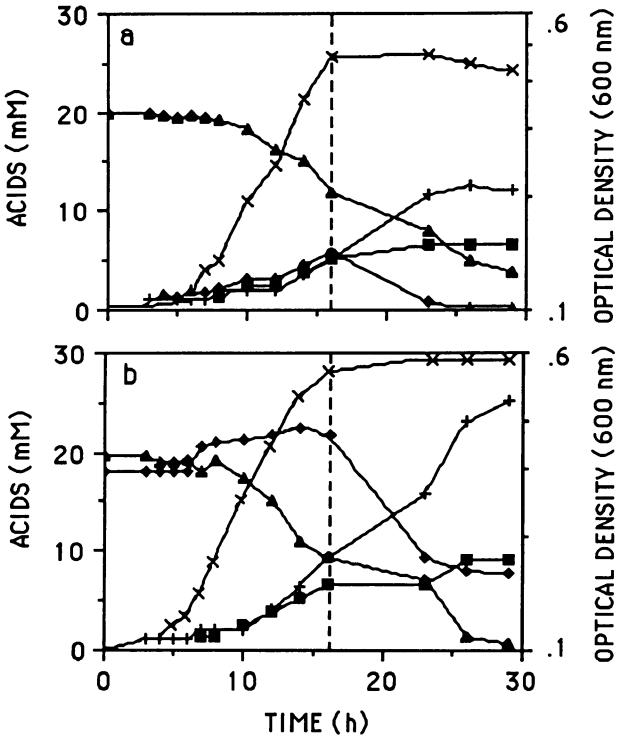

FIG. 2. Growth (×), succinate utilization (◆), and fermentation acid production (acetate [■] and propionate [+]) by strain H18 cultures provided with lactate (▲). Exogenous succinate (20 mM) was added to the culture (b).

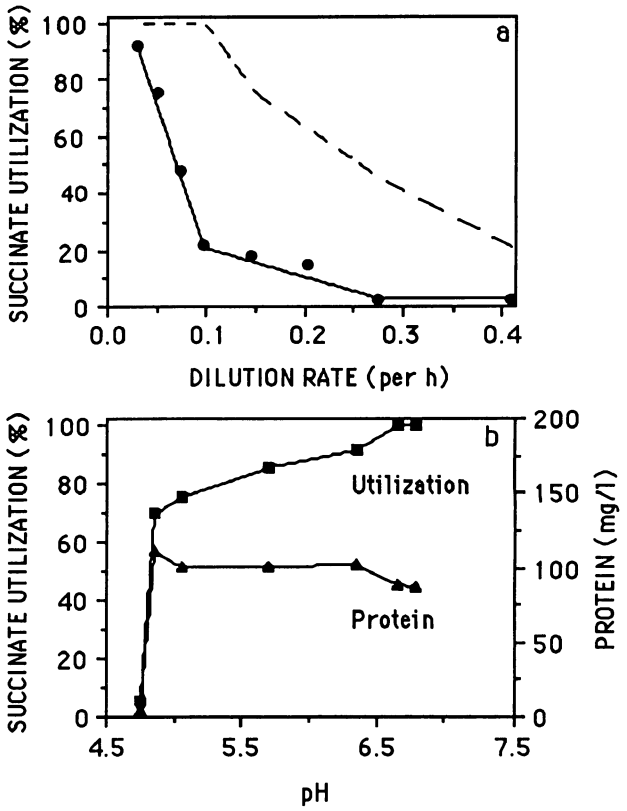

FIG. 3. Succinate utilization by strain H18 in glucose-limited (11 mM) continuous culture supplemented with 5 mM succinate and the effects of varying the dilution rate (a) and decreasing the pH (b) on succinate utilization at a dilution rate of 0.05 h^{-1}. The dashed line in panel a represents the theoretical consumption of succinate by nonenergized stationary-phase cultures (130 nmol min^{-1} mg of $protein^{-1}$).

Succinate transport. Glucose-energized, lactate-producing cultures were unable to transport [^{14}C]succinate (Fig. 4a). Although growth ceased when glucose was depleted, there was little change in ZΔpH, membrane potential (Δψ), total proton motive force (Δp), or ATP levels (Fig. 4b). Washed cells which were incubated in the absence of lactate had a similar Δp and ATP level (data not shown) and transported succinate rapidly (Fig. 5a). However, cells which were deenergized with 2-DG (30 min) took up little succinate. When washed cells were energized with glucose, succinate transport was greatly diminished, and more than 80% of the loss in transport activity occurred within 60 s (Fig. 5b). Similar results were noted if cells were energized with xylose (data not shown). Nonenergized and glucose-energized cells had similar concentrations of pyruvate, ATP, glucose-6-phosphate, and fructose-6-phosphate (Table 1). However, glucose-energized cells had 30-fold more fructose-1,6-bisphosphate and much less succinate than nonenergized cells.

When nonenergized cells were incubated with valinomycin, an ionophore which destroys the Δψ, or with 2-heptyl-4-hydroxy-quinoline *N*-oxide, an inhibitor of membrane-

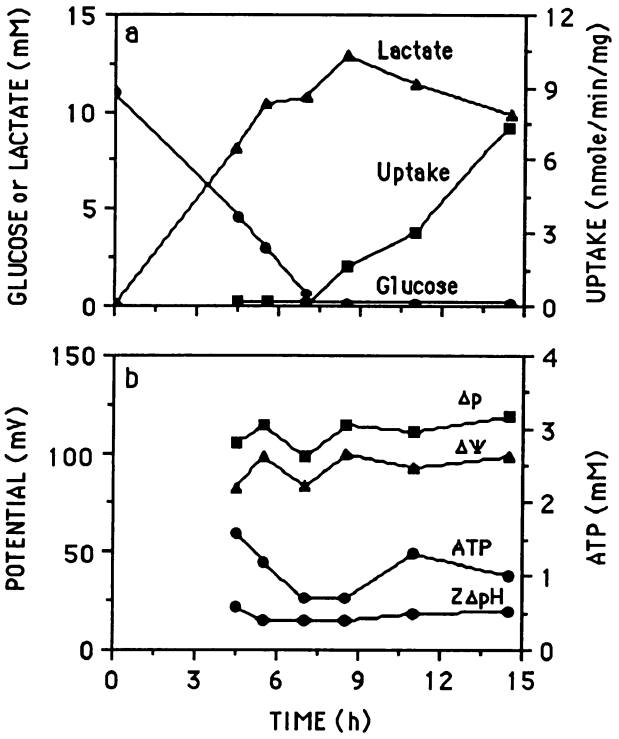

FIG. 4. Succinate transport, intracellular ATP, and proton motive force during growth on glucose. Succinate transport was determined by incubating a 200-μl sample of culture with 100 nCi of [^{14}C]succinate for 10 s and then filtering and washing the sample.

bound fumarate reductases, there was little effect on succinate uptake (Table 2). The H^+-ATPase inhibitor dicyclohexylcarbodiimide caused a 50% reduction in succinate transport. Iodoacetate, an inhibitor of glycolysis, 2-deoxyglucose, and the protonophore tetrachlorosalianilide greatly reduced succinate uptake. Nigericin and monensin, ionophores which decrease the ΔpH, virtually eliminated succinate transport.

When deenergized cells were loaded with potassium acetate and diluted into a buffer lacking acetate to create an artificial ΔpH, rapid rates of succinate transport were observed (Fig. 6). However, the imposition of an artificial Δψ (valinomycin-treated cells loaded with potassium and diluted into sodium phosphate) failed to drive succinate accumulation. Transport was optimal at pH 7.0, and uptake was reduced as the assay pH declined (Fig. 7a). An Eadie-Hofstee plot of succinate transport indicated that there was a disproportionately high rate of uptake at high substrate concentrations (Fig. 7b), but the low-velocity, high-affinity system could explain succinate utilization by whole cells.

The high-affinity transport system was sensitive to variations in osmolarity (Fig. 8). Even if only small amounts of KCl, NaCl, choline chloride, or sorbitol were added to the assay buffer (50 mM MES, 50 mM MOPS, 10 mM $MgCl_2$; adjusted to pH 7.0 with choline base), there was a dramatic decline in succinate uptake. When the assay buffer contained 2.5 mM K_2HPO_4 and the MES-MOPS-choline was decreased from 50 to 0 mM, there was no effect on transport (data not shown).

DISCUSSION

Michel and Macy (23) recently reported that *S. ruminantium* HD4 had a sodium-dependent succinate efflux mechanism which could also operate as an electrogenic uptake system. However, there was little evidence that this system was either sodium dependent or electrogenic. (i) Whole cells and membrane vesicles took up succinate in buffers that apparently lacked sodium. (ii) The electrogenic succinate transporter could not be driven by an artificial Δψ alone even if sodium was present. (iii) Although succinate efflux from right-side-out vesicles appeared to generate a Δψ, the assay buffer did not contain sodium. We found no evidence that either sodium or Δψ was involved in succinate transport by strain H18.

One could argue that strain HD4 differs from the H18 strain with respect to the mechanism of succinate uptake. However, our preliminary experiments with strain HD4 indicated that succinate transport was not stimulated by sodium. Michel and Macy routinely used higher succinate concentrations than we usually did (200 versus 4.55 μM, respectively), but succinate transport by strain H18 was not stimulated by sodium even at high substrate concentrations.

S. ruminantium HD4 transported succinate in the absence of an exogenous energy source (23), and we noted similar results with strains HD4 and H18. Since deenergization with 2-DG or iodoacetate greatly reduced succinate transport (Table 2), it appeared that energy derived from endogenous metabolism provided the driving force. Wallace (35) found that *S. ruminantium* contained as much as 48% glycogen and

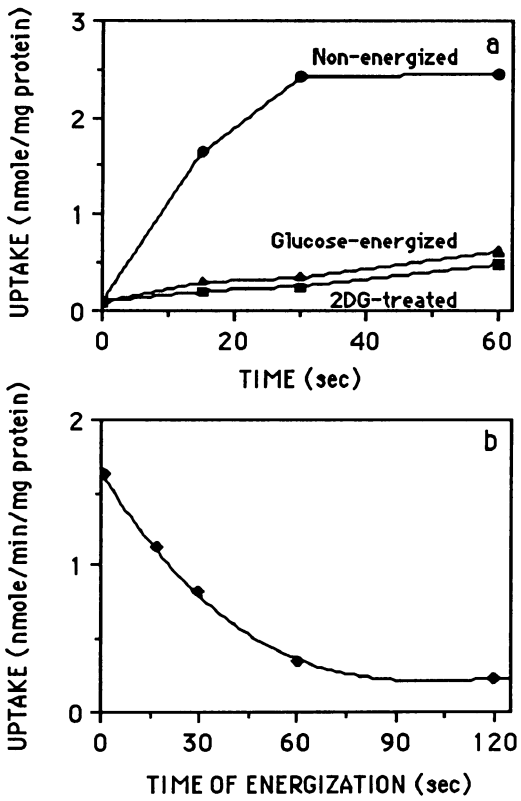

FIG. 5. Succinate transport by stationary-phase, nonenergized cells, cells which were energized with glucose (20 mM, 5 min), or cells which were deenergized with 2-DG (11 mM, 30 min) (a) and the rate at which glucose (20 mM) inhibited succinate transport (b).

TABLE 1. Intracellular metabolite pools of nonenergized and glucose-energized cells

Metabolite	Intracellular pools (mM)	
	Nonenergized	Glucose-energized[a]
Pyruvate	0.9	0.9
ATP	1.4	1.6
Glucose-6-phosphate	2.9	2.6
Fructose-6-phosphate	1.1	0.4
Succinate	15.0	ND[b]
Fructose-1,6-bisphosphate	0.9	28.1

[a] Cells were energized 5 min at 39°C with 20 mM glucose prior to extraction.
[b] ND, Not detected.

that 50% of this glycogen was used after 3 h of energy starvation. Nonenergized and glucose-energized cells of strain H18 had similar ATP levels and Δp, and this observation was consistent with the idea that nonenergized cells were metabolizing glycogen. Deenergized cells (2-DG or iodoacetate treated) had low Δp and ATP levels (data not shown).

When exponential cultures of strain H18 were washed and incubated in medium lacking glucose (or lactate), there was a rapid decrease in optical density (data not shown). The studies of Mink and Hespell (24) indicated that *S. ruminantium* D showed a marked loss of viability even after brief periods of starvation; decreases in viability were correlated with high rates of endogenous metabolism and a decline of cellular carbohydrate. The maintenance of a high Δp during periods of nutrient starvation may be responsible for the high rates of endogenous metabolism and losses of viability. Strain H18 maintains a high Δp in the absence of exogenous energy sources, but *Streptococcus bovis*, a ruminal bacterium which is very resistant to nutrient starvation, allows Δp to drop soon after glucose is depleted (27a).

In order to more precisely define the driving force for succinate transport, cells were deenergized with 2-DG, and an artificial Δψ or ΔpH was generated by potassium or acetate diffusion, respectively. An artificial Δψ drove tetraphenylphosphonium bromide uptake (data not shown), but it could not drive succinate transport (Fig. 6). This result was consistent with the observation that valinomycin, an ionophore which reduced the membrane potential, had little effect on uptake by nonenergized cells (Table 2). Because Δψ could not serve as a driving force for succinate transport, it

TABLE 2. Effects of metabolic inhibitors on succinate transport by nonenergized cells

Treatment[a] (concn)	Inhibition of transport (%)[b]
Valinomycin (10 μM)	21
HQNO (20 μM)	27
DCCD (0.25 μmol/mg of protein)	36
Iodoacetate (500 μM)	87
2-DG (20 mM)	89
TCS (10 μM)	90
Nigericin (10 μM)	92
Monensin (10 μM)	95

[a] HQNO, 2-Heptyl-4-hydroxy-quinolone *N*-oxide; DCCD, dicyclohexylcarbodiimide; TCS, tetrachlorosalianilide. Treatments were added 5 min prior to the addition of succinate, except iodoacetate and 2-DG, which were added 30 min prior to succinate.
[b] Based on a rate of 9.6 nmol min^{-1} mg of $protein^{-1}$.

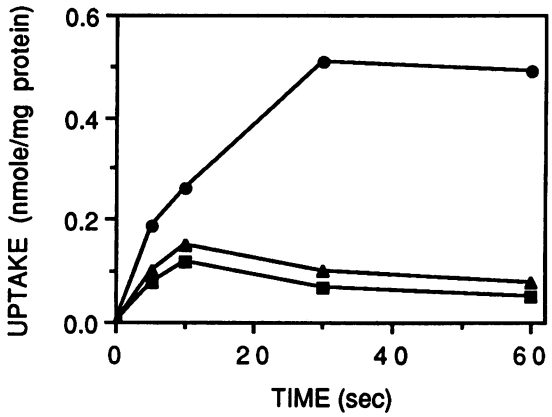

FIG. 6. Effects of artificial ΔpH or Δψ on succinate transport. Deenergized cells were loaded with 50 mM potassium acetate and diluted 50-fold into potassium phosphate to create an artificial ΔpH (●). Potassium acetate-loaded cells diluted into potassium acetate were used as controls (■). A Δψ was imposed by diluting valinomycin-treated (10 μM, 45 min, 0°C), potassium-loaded cells into sodium phosphate (▲).

appeared that the overall process was electroneutral. An artificial pH caused succinate accumulation and dissipation of the pH by tetrachlorosalianilide, and nigericin or monensin greatly inhibited transport. Since this dicarboxylic acid is nearly (>96%) fully dissociated at pH 7.0, $succinate^{2-}$ was apparently taken up electroneutrally in symport with two protons. Gutowski and Rosenberg (8) noted that succinate transport by *Escherichia coli* was accompanied by the uptake of two protons, and similar transport mechanisms have been found in *Bacillus subtilis* (21), *Pseudomonas putida* (7), *Salmonella typhimurium* (15), and a variety of other bacteria (16).

When strain H18 was incubated with various concentrations of [^{14}C]succinate, the transport kinetics were biphasic (Fig. 7b). Similar results were noted with *S. typhimurium* (15). Biphasic uptake had often been attributed to separate transport systems, but different conformations (high and low affinity) of the same transport protein could give similar kinetics. Since the high-affinity transport system could explain succinate utilization by intact cells, the function of the low-affinity system was not immediately apparent. Because lactate-grown cells of strain H18 produced succinate early in the incubation (Fig. 2), low-affinity transport may serve a role in succinate efflux.

Blackburn and Hungate (3) estimated that the ruminal concentration of succinate was approximately 4 μM and that this pool turned over at a rate of 10/min. On the basis of a bacterial succinate concentration of 3,000 mg/liter (5), the decarboxylation rate would be 13.3 nmol min^{-1} mg of $protein^{-1}$. In our experiments, mixed ruminal bacteria and strain H18 had rates of 100 and 130 nmol min^{-1} mg of $protein^{-1}$, respectively, but in these cases succinate was provided at 20 mM. The high-affinity succinate transport system had a velocity of 100 nmol min^{-1} mg of $protein^{-1}$, but the transport rate was only 7.5 nmol min^{-1} mg of $protein^{-1}$ when 4.5 μM succinate was added. Although this latter rate approached the in vivo rate of mixed cultures (7.5 versus 13.3 nmol min^{-1} mg of $protein^{-1}$), one should realize that *S. ruminantium* is not the only bacterium in the rumen. Even though strain H18 transported and decarboxylated

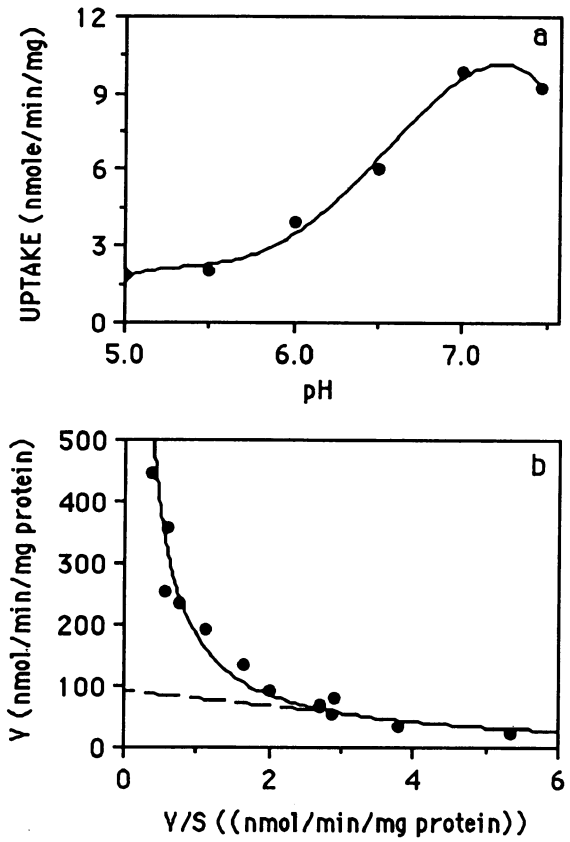

FIG. 7. Effects of pH on succinate uptake (a). Cells were incubated for 5 min in 50 mM MES–50 mM MOPS–10 mM $MgCl_2$ of various pHs before the addition of succinate. Eadie-Hofstee plot of succinate uptake (b). Succinate levels were varied from 2.7 to 1,200 μM. The dotted line is an extrapolation of the high-affinity transport system to the maximum theoretical velocity.

succinate much faster than strain HD4, there must be even more highly active organisms in the rumen.

Previous workers (29) used stationary cultures of *S. ruminantium* to study succinate metabolism and did not consider the effects of carbohydrate fermentation. When strain H18 was fermenting glucose or xylose, succinate was not decarboxylated or transported (Fig. 1, 4, and 5). Since nonenergized and glucose-energized cells had similar ΔpHs, this inhibition could not be explained by a decrease in driving force. *S. ruminantium* uses the phosphotransferase system (20) to take up glucose, but xylose is not transported by a mechanism involving group translocation (36). Glucose-depleted cultures fermented lactate and succinate simultaneously (Fig. 1 and 2), but growth was not observed. If cultures were initially provided with lactate, succinate was produced rather than utilized and this succinate was not used until growth ceased. Continuous culture studies indicated that even slow rates of glucose metabolism could inhibit succinate utilization.

Because sugar-dependent inhibition of succinate transport occurred almost immediately (Fig. 5b), it appeared that accumulation of an intracellular intermediate might be responsible. Succinate could not be detected in glucose-energized cells, and thus internal succinate accumulation could not account for the inhibition of transport (Table 1). There were no significant changes in pyruvate, glucose-6-phosphate, or fructose-6-phosphate after glucose addition. Fructose-1,6-bisphosphate levels increased dramatically, but the implications of this change are uncertain.

When strain H18 was provided with an excess of glucose, lactate was the primary fermentation product. However, at slow growth rates, the organism shifted to a fermentation consisting of acetate and propionate. Similar results have been reported for other strains of *S. ruminantium* (27, 33). Wallace (33) noted that the lactate dehydrogenase (LDH) of *S. ruminantium* was homotropically activated by pyruvate and used this observation to explain the shift from acetate to lactate production at fast growth rates. In *S. bovis* (28), *Butyrivibrio fibrisolvens* (32), and *Lactobacillus casei* (6), LDH was activated by fructose-1,6-biphosphate, but the *S. ruminantium* LDH was fructose-1,6-bisphosphate independent (33). The LDH of strain H18 did not respond to fructose-1,6-bisphosphate (data not shown), but there was no increase in pyruvate concentrations if cells were energized with glucose (Table 1). On the basis of these results, it did not appear that an increase in intracellular pyruvate could explain the shift to lactate production.

Even small increases in osmolarity decreased succinate transport by strain H18 (Fig. 8). It has generally been assumed that bacteria maintain relatively high concentrations of internal solute and that cell volume is determined by the rigid layer of peptidoglycan. Recent work has shown that some transport proteins respond to changes in external ion concentration (34), but these transport systems are themselves involved in the regulation of turgor and respond in a positive fashion to increasing external salt. The mechanism underlying the effects of turgor on bacterial transport has not been thoroughly elucidated.

Given the facts that strain H18 lyses easily and that *S. ruminantium* often forms spheroplasts during nutrient starvation (17), it is possible that glucose energization could mediate a decrease in turgor and cell shrinkage. In *Mycoplasmsa gallisepticum*, glucose addition causes cell shrinkage via a mechanism involving sodium expulsion (18). The structure and orientation of the *Selenomonas* succinate transport system are not known, but in *E. coli*, periplasmic proteins have been implicated (2). An interesting but yet unproven scheme of regulation for the selenomonad succinate transport system might entail (i) cell swelling resulting

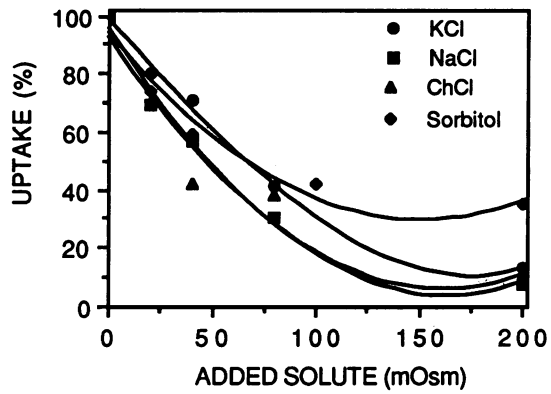

FIG. 8. Effects of increasing extracellular osmotic strength on succinate uptake by nonenergized strain H18 cells. Cultures were washed and incubated in 50 mM MOPS–50 mM MES–10 mM $MgCl_2$ (pH 7.0). Chloride salts or sorbitol was added 5 min prior to succinate. All values were compared with a control rate of 9.7 nmol min^{-1} mg of $protein^{-1}$.

in a closer and more functional orientation of the membrane transport protein to periplasmic-binding proteins during periods of energy starvation or (ii) a reduction of turgor after glucose energization and separation of membrane transport proteins from periplasmic components.

Although the inhibition of succinate utilization by carbohydrate and osmotic strength may be interesting from a mechanistic standpoint, these effects probably have little direct effect on succinate cross-feeding in vivo. The osmolarity of ruminal fluid is tightly controlled (14). Soluble carbohydrates can accumulate soon after feeding, and *S. ruminantium* would be unable to utilize succinate during this period. However, during most of the feeding cycle, the rumen operates as a slow-dilution-rate chemostat in which growth rate is limited by carbohydrate availability.

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