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Generation of a Membrane Potential by Sodium-Dependent Succinate Efflux in *Selenomonas ruminantium*

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When *Selenomonas ruminantium* HD4 was grown in a chemostat, maximal succinate production and the highest molar growth yield values were both observed at a dilution rate of roughly 0.2 h^{-1}. To determine the possible relationship between succinate efflux and high molar growth yields, the generation of a membrane potential by succinate efflux was studied in whole cells and vesicles (inside-out and right-side-out) prepared from *S. ruminantium*. Washed whole cells took up succinate in the absence of an exogenous energy supply; uptake was completely abolished by brief treatment with dinitrophenol or with nigericin and valinomycin. High levels of sodium ions (with respect to the intracellular sodium concentration in the assay buffer had a stimulatory effect on succinate uptake. When succinate was added to inside-out vesicles, a membrane potential (inside positive) was generated, as indicated by fluorescence quenching of the anionic lipophilic dye Oxonol V. Fluorescence quenching was sensitive to uncoupling by gramicidin D but only partially sensitive to the uncoupler carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone. In right-side-out vesicles, succinate uptake could be driven by an artificially imposed sodium gradient but not by a potassium diffusion potential; imposition of both a sodium gradient and potassium diffusion potential resulted in improved succinate uptake. The generation of a membrane potential (inside negative) upon succinate efflux was demonstrated directly in right-side-out vesicles when succinate-loaded vesicles were diluted into succinate-free buffer, and the lipophilic cationic probe tetraphenylphosphonium accumulated in the vesicles. Results indicate that an electrogenic succinate-sodium symporter is present in *S. ruminantium*. Transport of succinate out of the cell via the symporter might be responsible for the high molar growth yields obtained by this organism when it is grown at dilution rates where maximal succinate production occurs.

When *Selenomonas ruminantium* HD4 is grown in glucose-limited continuous culture, succinate is a major product formed only at dilution rates ranging from 0.10 to 0.30 h^{-1} (10–12). The maximal molar growth yield (80 g [dry weight] of cells per mol of glucose) was observed at the dilution rate (0.20 h^{-1}) at which succinate production was also at its highest level.

In an attempt to explain the high molar growth yields observed for *S. ruminantium*, we investigated the possibility that a "new" energy-yielding mechanism might be associated with succinate production in this organism. In 1979 Michels et al. (13) proposed a mechanism for energy conservation in bacteria whereby carrier-mediated efflux of metabolic endproducts in symport with protons could potentially lead to the generation of an electrochemical proton gradient. Such a proton gradient could then be used either directly to perform metabolic work or to synthesize ATP via a reversible ATPase. In homolactic streptococci and in *Escherichia coli* the generation of such a proton gradient by lactate efflux is supported by firm experimental evidence (6, 14, 17, 20–22). If carrier-mediated end-product efflux of succinate also exists in *S. ruminantium*, then this form of energy conservation might contribute significantly to the very high yields observed when succinate is a major product of glucose fermentation. In this report we describe the generation of a membrane potential by succinate efflux in *S. ruminantium*. Our results also indicate that this electrogenic succinate efflux is sodium rather than proton dependent. Energy conservation by carrier-mediated efflux of a fermentation product other than lactate had not previously been demonstrated.

MATERIALS AND METHODS

Strain HD4 of *S. ruminantium*, culture medium, and conditions for continuous culture have been described previously (10, 11).

Analytical methods. Molar growth yield values were calculated as described by Stouthamer and Bettenhausen (19). Cell dry weights were determined by the method of Isaacson et al. (4). Amounts of glucose, lactate, and succinate were determined enzymatically as recommended by Boehringer-Mannheim Biochemicals (Indianapolis, Ind.; Methods of Enzymatic Food Analysis). Volatile fatty acids were estimated as reported previously (7). When it was necessary to increase the steady-state succinate concentration in continuous culture, exogenous succinate was added to the reservoir of in-flowing medium. The amount of succinate made by cells under such conditions was calculated by determining the difference between total steady-state succinate concentration in culture supernatants and that present in the medium reservoir.

Deenergization of cells. Cell suspensions (10 mg [dry weight] per ml) were incubated in TM buffer [5 mM Tris hydrochloride, 5 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 7.2), 100 mM choline chloride, 0.2 mM potassium chloride, 1 mM magnesium chloride, 2 mM dithiothreitol] with shaking in the presence of 5 mM dinitrophenol for 45 s. After deenergization, cells were centrifuged, washed four times with an equal volume of TM buffer, and concentrated to yield approximately 75 mg (dry weight) per ml.

Succinate uptake assays. Uptake of [1,4-^{14}C]succinate into whole cells or right-side-out vesicles was measured in a time

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course assay by the filtration technique of Otto et al. (14). Assays were carried out under aerobic conditions. Unless otherwise stated, reaction mixtures contained 200 μM [1,4-^{14}C]succinate (specific activity, 0.5 μCi μmol^{-1}). Uptake was stopped by rapid dilution of 100-μl samples in 3 ml of 0.2 M lithium chloride. Samples were filtered on 25-mm membrane filters (type HA, 0.45-μm pore size; Millipore Corp., Bedford, Mass.). Filters were washed twice with an equal volume of stopping solution, dried at 39°C, and counted by liquid scintillation.

Preparation of whole cells loaded with succinate. Cells (approximately 25 mg of protein per ml) were loaded with succinate by incubation for several hours at room temperature in TM buffer containing 50 mM succinate choline (pH 6.6). After incubation, cells were centrifuged and suspended to a final concentration of approximately 75 mg of protein per ml in TM buffer containing 50 mM choline succinate (pH 7.2).

Preparation of inside-out vesicles. Cells were harvested by centrifugation, washed twice with 50 mM TM buffer (pH 7.2), and suspended (1 g [wet weight] of cells in 5 ml of buffer) in 50 mM MOPS [3-(*N*-morpholino)propanesulfonic acid (pH 7.0)], 5 mM magnesium chloride, and 100 mM sucrose. Cells were broken by one passage through an Aminco French press at 22,000 lb in^{-2}. The lysate was centrifuged at 10,000 × *g* for 15 min to remove unbroken cells. The supernatant was centrifuged at 100,000 × *g* for 60 min. The membrane pellet was washed once and suspended to a final protein concentration of 12.5 mg of protein per ml. Vesicle suspensions were stored in liquid nitrogen until use. The protein content of these vesicles was determined by the method of Markwell et al. (9).

Fluorescence quenching of Oxonol V by ATP hydrolysis and succinate uptake. Changes in membrane potential in inside-out vesicles were followed by measuring fluorescence quenching of the lipophilic anionic dye Oxonol V (18, 23); the reaction was followed in 2-ml quartz cuvettes using an Aminco Bowman spectrofluorometer. Excitation and emission wavelengths were 580 and 650 nm, respectively. Vesicles were suspended (0.2 mg of protein per ml) in 50 mM MOPS (pH 7.0) containing 5 mM magnesium chloride and 100 mM sucrose at 25°C. Oxonol V, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), carbonyl cyanide-*m*-chlorophenylhydrazone, and dicyclohexylcarbodiimide (DCCD) were dissolved in 100% methanol and added to the reaction mixtures at a final methanol concentration of 1%. The final concentration of Oxonol V was 1 μM.

Preparation of right-side-out vesicles. Right-side-out vesicles were prepared from *S. ruminantium* as described by Kaback (5), with the following modifications. Mutanolysin (30,000 U per 2 g [wet weight] of cells) was substituted for lysozyme, and EDTA was replaced by nitrilotriacetic acid; the incubation with mutanolysin was performed on ice rather than at room temperature (T. A. Michel and J. M. Macy, J. Microbiol. Methods, in press).

Loading of right-side-out vesicles. To load right-side-out vesicles with succinate, a suspension of vesicles (5 mg of vesicle protein per ml) in 50 mM potassium succinate (pH 6.6) was incubated on ice with occasional shaking for 60 min. After centrifugation, the pellet was washed twice and suspended in 50 mM potassium succinate (pH 7.2) at a concentration of 30 mg of protein per ml. Control vesicles (i.e., not succinate loaded) were incubated in 50 mM potassium phosphate (pH 6.6) and suspended in 50 mM potassium phosphate (pH 7.2). Vesicles (30 mg of protein per ml) were loaded with potassium or sodium ions or both by incubating them for 30 min on ice with 100 mM potassium phosphate, 100 mM sodium phosphate, or both in the presence of 2 μM valinomycin.

Uptake of Ph_4P^+ by succinate efflux. Uptake of U-^{3}H-labeled tetraphenylphosphonium ([U-^{3}H]Ph_4P^+) was assayed as described above for succinate uptake. Succinate-loaded vesicles were diluted 100-fold into 50 mM potassium phosphate buffer (pH 7.2) or into 50 mM potassium phosphate buffer supplemented with 50 mM potassium succinate (pH 7.2). The concentration of [U-^{3}H]Ph_4P^+ (specific activity, 26 Ci mmol^{-1}) in the assay mixture was 0.4 μM. To correct for nonspecific Ph_4P^+ binding, average filter counts obtained in control experiments, in which potassium-loaded vesicles (30 mg of protein per ml) were diluted 100-fold into 50 mM lithium chloride, were subtracted from filter counts for experiments with succinate-loaded vesicles.

Uptake of succinate driven by artificially imposed ion gradients. Succinate uptake was measured as described above for whole cells, except that 200-μl samples were filtered. Potassium-loaded right-side-out vesicles (30 mg of protein per ml) were diluted 100-fold into 50 mM potassium phosphate–50 mM sodium phosphate buffer (pH 7.2; $\Delta\mu_{Na^+}$ only) or into 50 mM sodium phosphate buffer (pH 7.2; $\Delta\psi$ and $\Delta\mu_{Na^+}$). Alternatively, potassium-sodium-loaded vesicles were diluted 100-fold into 50 mM sodium phosphate buffer (pH 7.2; $\Delta\psi$ only).

Materials. [1,4-^{14}C]Succinate and [U-^{3}H]Ph_4P^+ were obtained from the Radiochemical Centre (Amersham, England). Oxonol V was a gift from A. B. Bennett, Mann Laboratories (University of California, Davis). All other reagents were from Sigma Chemical Co. (St. Louis, Mo.) or were of comparable analytical grade.

RESULTS

Experiments with growing cells. When *S. ruminantium* HD4 was grown in glucose-limited continuous culture (11), maximum molar growth yield values and highest amounts of succinate produced were both observed at a dilution rate of roughly 0.20 h^{-1}. In Table 1 are shown the relative amounts of fermentation products made at each dilution rate examined as well as the total number of ATP equivalents (based on all known energy-conserving reactions; efflux-mediated ion gradients are not included) that could have been generated during the formation of these products.

The effect of a decrease in the succinate concentration gradient (inside relative to outside of the cell) on the molar growth yield was studied by increasing the effective succinate concentration in the growth medium (Fig. 1). An increasing extracellular succinate concentration (i.e., decreasing succinate concentration gradient) resulted in lowered molar growth yields (Fig. 1). Relative steady-state levels of fermentation end products, measured at each level of exogenous succinate, are shown in Fig. 1B. A change in relative amounts of fermentation products was observed when the exogenous succinate concentration was 0.35 mM; higher concentrations of exogenous succinate did not result in any further changes.

Experiments with whole cell suspensions. In the absence of an exogenous energy supply, whole cell suspensions of *S. ruminantium* rapidly transported succinate into the cell (Fig. 2). When cell suspensions were deenergized by treatment with dinitrophenol for 45 s, active transport of succinate was completely abolished (Fig. 2). In the presence of potassium ions (provided by the assay medium), a combination of the ionophores nigericin and valinomycin caused a total collapse

TABLE 1. Relative amounts of fermentation products made and total amount of ATP generated by chemostat-grown *S. ruminantium* HD4 as a function of dilution rate

Product[a]	Amt generated (mmol of acid per mmol of glucose) at the following dilution rates (h^{-1}):						
	0.07	0.09	0.12	0.19	0.32	0.52	0.62
Lactate (1)	0.09	0.17	0.13	0.31	1.17	1.41	1.60
Acetate (2)	1.18	0.96	0.71	0.70	0.63	0.49	0.40
Propionate (2.66)	1.01	1.18	0.78	0.36	0.32	0.35	0.24
Succinate (2)	0.06	0.04	0.10	0.61	0.21	0.24	0.13
Total ATP[b]	5.26	5.31	3.82	3.89	3.70	3.80	3.30

[a] Numbers within parentheses indicate how many total ATP equivalents arise from the formation of 1 mol of each fermentation end product formed.

[b] The total numbers of ATP equivalents generated, based on amounts of products formed and the number of maximal ATP equivalents per mole of substrate arising from each energy-conserving reaction are as follows (11): pyruvate kinse, 1; phosphoenolpyruvate carboxykinase, 1; acetate thiokinase, 1; fumarate reductase, 1; methylmalonyl coenzyme A decarboxylase, 0.66. ATP equivalents generated by end-product efflux are not included.

of the membrane potential ($\Delta\psi$) and ΔpH and abolished uptake completely; uptake was not abolished by preincubation of cells with 2 mM isopropylthiogalactoside or 1 mM iodoacetic acid (data not shown).

After cells were washed in 20 μM sodium-containing buffer and uptake experiments were performed in a buffer containing 15.8 mM sodium, improved rates of succinate uptake and a higher final degree of accumulation were observed (data not shown).

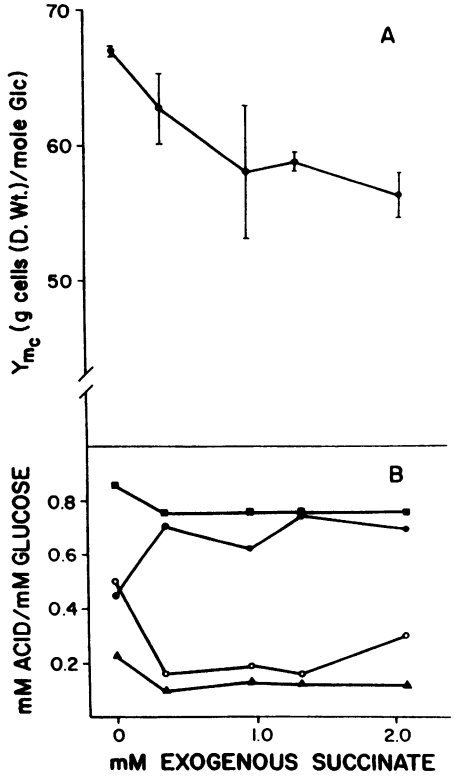

FIG. 1. Effect of the addition of exogenous succinate to the in-flowing medium of *S. ruminantium* HD4 continuous cultures (A) on the molar growth yield and (B) on the steady-state levels of the following fermentation end products: (■) acetate, (●) propionate, (▲) lactate, (○) succinate. Bars in A represent standard deviations of the data (n = 3).

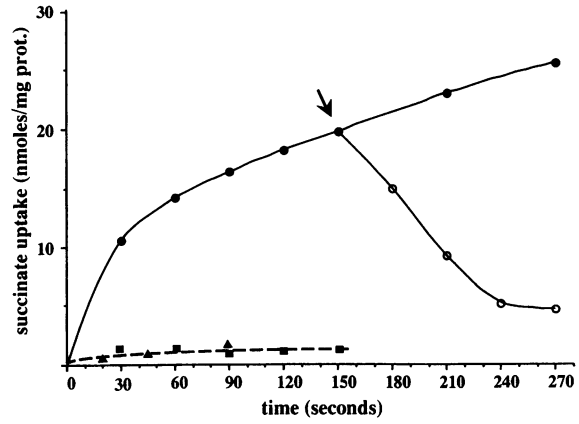

FIG. 2. Time course of [1,4-^{14}C]succinate uptake into whole cells of *S. ruminantium* HD4 at pH 7.2 and 25°C: (●) uptake driven in the absence of exogenous energy supply; (○) in a parallel experiment, at the time indicated by the arrow, 20 mM (final concentration) nonradioactive succinate choline was added to the assay mixture; (■) uptake after preincubation of cells with 2 mM dinitrophenol for 45 s; (▲) uptake in the presence of 0.2 μM nigericin and 2.0 μM valinomycin. Cells were diluted into assay buffer to a concentration of 0.25 mg of protein per ml.

Figure 2 also shows radiolabeled succinate uptake into whole cells with time as effected by a chase with unlabeled succinate. The chase with unlabeled succinate molecules caused a rapid efflux of the previously accumulated labeled succinate molecules. Labeled succinate was also transported into cells that had been preloaded with 20 mM succinate (data not shown), i.e., against a concentration gradient (counterflow).

Experiments with vesicles. (i) Inside-out vesicles. When ATP was added to a suspension of inside-out vesicles prepared from *S. ruminantium*, 20% quenching of the fluorescence of Oxonol V was observed (Fig. 3A). Baseline levels of fluorescence, equal to those observed before ATP addition, were not recovered when the uncoupler FCCP was subsequently added (Fig. 3A). Fluorescence quenching was likewise insensitive to the addition of the ATPase inhibitor DCCD (Fig. 3D). Baseline levels of fluorescence could be completely restored, however, by the channel-forming ionophore gramicidin D (Fig. 3C). When the vesicle preparation was preincubated with the ionophore monensin, fluorescence quenching by ATP was partially sensitive to FCCP (Fig. 3B) and DCCD (Fig. 3E).

As with ATP, slightly more than a 20% decrease in fluorescence was observed when succinate-choline was added to the vesicle suspension (Fig. 4A). The fluorescence quenching, as with ATP, was insensitive to the addition of FCCP (Fig. 4A) but sensitive to gramicidin D (Fig. 4C). Pretreatment of the vesicles with monensin also partially restored sensitivity of fluorescence quenching to FCCP (Fig. 4B).

(ii) Right-side-out vesicles. The establishment of a membrane potential by succinate efflux was directly demonstrated by measuring uptake of Ph_4P^+ into right-side-out vesicles that had been loaded with 50 mM succinate (Fig. 5). When such vesicles were diluted into succinate-free potassium phosphate buffer, rapid uptake of the labeled probe was observed. After 15 s, Ph_4P^+ had accumulated inside these vesicles roughly 12-fold; thus succinate efflux resulted in the transient generation of a membrane potential (inside negative) of roughly 65 mV. Dilution of succinate-loaded vesicles

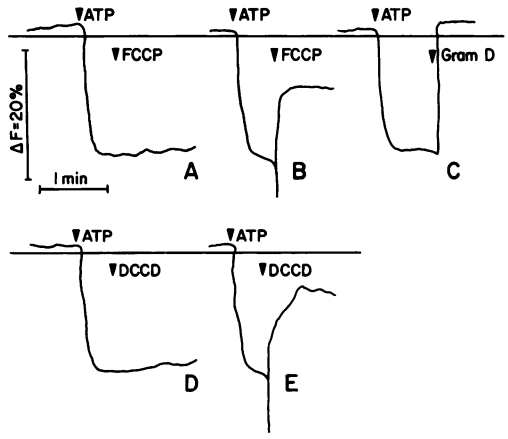

FIG. 3. Fluorescence quenching of the dye Oxonol V in inside-out membrane vesicles from *S. ruminantium* HD4. Representative tracings of the chart recordings are shown. Inside-out vesicles prepared from *S. ruminantium* were suspended in 2 ml of 50 mM MOPS (pH 7.0)–5 mM $MgCl_2$–100 mM sucrose at 25°C. The vesicle concentration was 0.2 mg of protein per ml. Reactions were started by adding 20 μl of 0.5 M ATP (first arrow). At the time indicated by the second arrow, 20 μl of 1 mM FCCP (A and B), 20 μl of 1 mM gramicidin D (C), or 20 μl of 1 mM DCCD (D and E) were added. The vesicles used in experiments B and E were first incubated for 60 min on ice in the presence of monensin (1 mM).

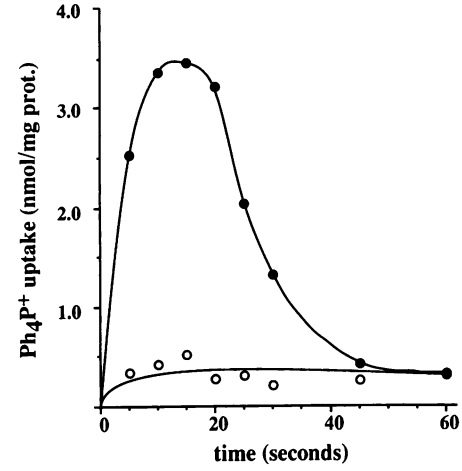

FIG. 5. Time course of Ph_4P^+ uptake driven by succinate efflux in *S. ruminantium* HD4 right-side-out membrane vesicles. Vesicles loaded with 50 mM potassium succinate were diluted 100-fold into (●) 50 mM potassium phosphate buffer (pH 7.2) at 25°C or (○) 50 mM potassium phosphate buffer (pH 7.2) supplemented with 50 mM potassium succinate at 25°C.

into potassium phosphate buffer supplemented with 50 mM potassium succinate did not result in significant accumulation of the probe (Fig. 5).

To establish more firmly the sodium dependency of the succinate carrier, we studied whether an artificially established membrane potential ($\Delta\psi$), sodium gradient ($\Delta\mu_{Na^+}$), or a combination of both could drive uptake of ^{14}C-labeled succinate into right-side-out vesicles (Fig. 6). Succinate uptake could be driven by a sodium gradient only (i.e., dilution of potassium-loaded vesicles into sodium-potassium phosphate buffer) and was improved when potassium-loaded vesicles were diluted into sodium phosphate buffer ($\Delta\psi$ and $\Delta\mu_{Na^+}$). Dilution of sodium-potassium-loaded vesicles into sodium phosphate buffer ($\Delta\psi$ only) did not result in succinate uptake beyond equilibrium values.

DISCUSSION

The changes in molar growth yields observed when *S. ruminantium* was grown in glucose-limited continuous culture at dilution rates between 0.10 and 0.30 h^{-1} could, in principle, be caused by either changes in Y_{ATP} (grams [dry weight] of cells per mole of ATP) or by increased ATP production. General arguments ruling out changing Y_{ATP} values have been presented elsewhere (11). Therefore, since the number of ATP produced via known energy-conserving reactions in *S. ruminantium* do not account for the increased amount of ATP that must have been formed (i.e., because of the increased molar growth yield) (Table 1), this organism must have an additional means of conserving energy.

Physiological results suggested that carrier-mediated succinate efflux is responsible for the increase in molar growth yield observed at the dilution rate of 0.20 h^{-1}, since partial collapse of the succinate gradient existing at this dilution rate resulted in decreased molar growth yields (Fig. 1A). The decrease could not be explained on the basis of a changed

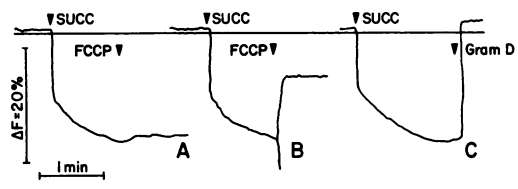

FIG. 4. Fluorescence quenching of Oxonol V in inside-out membrane vesicles from *S. ruminantium* HD4. Representative tracings of the chart recordings are shown. Vesicles were suspended in 2 ml of 50 mM MOPS (pH 7.0)–5 mM $MgCl_2$–100 mM sucrose at 25°C. The vesicle concentration was 0.2 mg of protein per ml. Reactions were started by adding 200 μl of 200 mM succinate choline (SUCC; first arrow). At the time indicated by the second arrow, 20 μl of 1 mM FCCP (A and B) or 20 μl of 1 mM gramicidin D (C) was added. The vesicles used in experiment B were first incubated for 60 min on ice in the presence of monensin (1 mM).

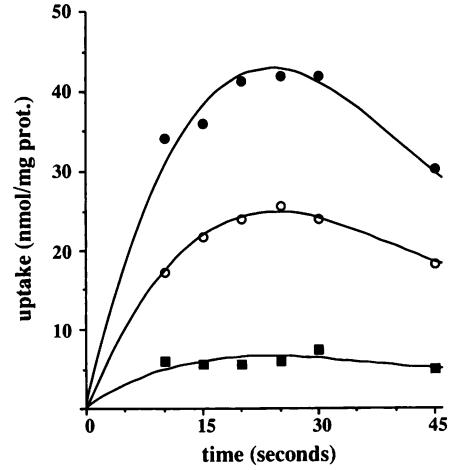

FIG. 6. Uptake of [1,4-^{14}C]succinate (specific activity, 1.8 μCi μmol^{-1}) into valinomycin-treated right-side-out vesicles loaded with (● and ○) 50 mM potassium phosphate or with (■) 50 mM potassium-sodium phosphate. These vesicles were diluted 100-fold into (● and ■) 50 mM sodium phosphate buffer (pH 7.2) or into (○) 50 mM sodium-potassium phosphate buffer (pH 7.2) at 25°C.

fermentation pattern, since, at increasing concentrations of exogenous succinate greater than 0.35 mM, the fermentation pattern remained unchanged, whereas molar growth yields continued to decrease (Fig. 1B).

Stoichiometric extrusion of ions in symport with succinate requires the existence of a reversible and electrogenic carrier in the cytoplasmic membrane. The results discussed below, describing the system for succinate transport in *S. ruminantium*, indicate that both of these requirements are met.

The existence of a reversible carrier, responsible for succinate translocation in the membrane of *S. ruminantium*, was demonstrated by the observation of counterflow (see Results) and by the chase experiment shown in Fig. 2 (8). In this latter experiment, the rapid efflux of previously accumulated radiolabeled succinate resulted from the competition for binding to the carrier (i.e., for entry and for reentry upon exit) of the labeled molecules with the unlabeled molecules added during the chase. Since in this experiment the competition for the carrier was occurring on the periplasmic side of the membrane (whereas in the counterflow experiment the opposite was the case), the demonstration of counterflow and of chase-mediated efflux complement each other and substantiate the reversible nature of the succinate carrier.

The abolition of succinate uptake by nigericin-valinomycin and the deenergization of transport by brief dinitrophenol treatment of whole cells provided the first indication that the succinate carrier in *S. ruminantium* operated electrogenically. The results obtained from experiments with inside-out and right-side-out vesicles support this conclusion, as discussed below.

The generation of a membrane potential (inside positive) by ATP hydrolysis in inside-out vesicles is well documented (15). In our experiments membrane potential generation by ATP hydrolysis was also observed, as evidenced by the fluorescence quenching of Oxonol V (Fig. 3). The finding that succinate uptake into inside-out vesicles prepared from *S. ruminantium* also resulted in fluorescence quenching of the dye (Fig. 4) indicated that succinate transport is electrogenic. It should be noted that the direction of succinate translocation by these inside-out vesicles is actually the physiological direction (i.e., the carrier is operating in the same direction as it does in vivo during succinate efflux out of the cell).

Experiments with right-side-out vesicles further corroborated the electrogenic nature of succinate efflux. With these vesicles it was possible to demonstrate directly the generation of a membrane potential upon succinate efflux (Fig. 5). Uptake of Ph_4P^+ was only observed when succinate-loaded vesicles were diluted into succinate-free buffer. No uptake was observed, however (after correcting for unspecific binding), when succinate-loaded vesicles were diluted into succinate-containing buffer (i.e., no net efflux of succinate, since inside and outside concentrations of this solute were identical), indicating that the generation of the membrane potential was solely due to succinate efflux.

The following experiments led to the conclusion that the membrane potential generated by succinate efflux involves sodium ions rather than protons. In succinate uptake studies with whole cells washed with low-sodium buffer, the presence of higher concentrations of sodium ions in the assay medium had a stimulatory effect on succinate uptake (see Results). The fluorescence quenching experiments shown in Fig. 4 further demonstrate that sodium rather than protons is the coupling ion for succinate transport. Baseline fluorescence could not be recovered after the addition of FCCP to succinate-treated vesicles but could be partially recovered by preincubation of vesicles with monensin (an ionophore with sodium-proton antiporter activity). The observation that baseline fluorescence was completely recovered when the channel-forming ionophore gramicidin D was added to succinate-treated vesicles (Fig. 4) is also consistent with sodium-dependent transport.

Further support for a sodium-coupled succinate transport system was provided by the results of the experiment shown in Fig. 6, with right-side-out vesicles. Succinate uptake in right-side-out vesicles was driven by an artificial sodium gradient ($\Delta\mu_{Na^+}$) but not by a potassium diffusion potential alone ($\Delta\psi$). The finding that a combination of both $\Delta\psi$ and $\Delta\mu_{Na^+}$ resulted in improved succinate uptake is possibly due to the net movement of total charge across the membrane, since the operation of the sodium-succinate symporter is electrogenic and generates an electric potential of polarity opposite to that of the potassium diffusion potential.

Why *S. ruminantium* switches from propionate to succinate production at certain dilution rates (roughly 0.2 h^{-1}) and why sodium rather than protons is the coupling ion in this latter energy-conserving reaction are both intriguing and perhaps related questions. Propionate production by *S. ruminantium* occurs from succinyl coenzyme A via a methylmalonyl coenzyme A decarboxylase (11). In *Veilonella alcalescens* this enzyme has been shown to be linked to the extrusion of sodium ions (2); whether this is also the case in *S. ruminantium* has not yet been investigated. The fact that succinate efflux and (by analogy) possibly also methylmalonyl coenzyme A decarboxylation result in the extrusion of sodium ions would indicate that sodium gradients are an important aspect of the bioenergetics of *S. ruminantium*, especially since most rumen organisms have a high requirement for sodium (1). Sodium-dependent uptake of amino acids was described recently for *Streptococcus bovis* (16).

The concentration of succinate in the rumen is very low, whereas the concentrations of propionate and sodium are usually quite high (3, 16). Because at certain rumen turnover rates, it might be difficult for *S. ruminantium* to maintain substantial sodium gradients by sodium-dependent propionate production, the switch from sodium-dependent propionate production to sodium-dependent extrusion of succinate observed in continuous culture might have evolved so that high succinate concentration gradients can drive sodium extrusion into an environment with high sodium and propionate concentrations.

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