

Cellodextrin Efflux by the Cellulolytic Ruminant Bacterium *Fibrobacter succinogenes* and Its Potential Role in the Growth of Nonadherent Bacteria

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When glucose or cellobiose was provided as an energy source for *Fibrobacter succinogenes*, there was a transient accumulation (as much as 0.4 mM hexose equivalent) of cellobiose or cellotriose, respectively, in the growth medium. Nongrowing cell suspensions converted cellobiose to cellotriose and longer-chain cellodextrins, and in this case the total cellodextrin concentration was as much as 20 mM (hexose equivalent). Because cell extracts of glucose- or cellobiose-grown cells cleaved cellobiose and cellotriose by phosphate-dependent reactions and glucose 1-phosphate was an end product, it appeared that cellodextrins were being produced by a reversible phosphorylase reaction. This conclusion was supported by the observation that the ratio of cellodextrins to cellobiose with one greater hexose [$n/(n + 1)$] was approximately 4, a value similar to the equilibrium constant (K_{eq}) of cellobiose phosphorylase (J. K. Alexander, *J. Bacteriol.* 81:903-910, 1961). When *F. succinogenes* was grown in a cellobiose-limited chemostat, cellobiose and cellotriose could both be detected, and the ratio of cellotriose to cellobiose was approximately 1 to 4. On the basis of these results, cellodextrin production is an equilibrium (mass action) function and not just an artifact of energy-rich cultural conditions. Cellodextrins could not be detected in low-dilution-rate, cellulose-limited continuous cultures, but these cultures had a large number of nonadherent cells. Because the nonadherent cells had a large reserve of polysaccharide and were observed at all stages of cell division, it appeared that they were utilizing cellodextrins as an energy source for growth. The noncellulolytic bacterium *Streptococcus bovis* persisted in batch culture with *F. succinogenes* even though cellulose was the only energy source, and the ratio of *S. bovis* to *F. succinogenes* was approximately 1 to 4. The carbohydrate metabolism of *F. succinogenes* seems to reflect a compromise between the energetic advantage of a phosphorylase reaction and the potential loss of carbon and energy as extracellular cellodextrins.

Ruminant animals have developed the capacity to digest cellulose by relying on a symbiotic relationship with cellulolytic bacteria (17). When Bryant and Burkey (6) enumerated ruminal bacteria from animals fed wheat straw, starch-digesting bacteria always outnumbered the cellulolytic bacteria. Cultural difficulties and attachment may have compromised the cellulolytic count, but this early work clearly indicated that nonfibrolytic bacteria could persist in the rumen even if the diet was primarily cellulose and hemicellulose.

In the 1940s, Hungate (17) isolated three cellulolytic species, and later studies indicated that these bacteria were the predominant cellulose-digesting bacteria in the rumen (6, 8). *Fibrobacter (Bacteroides) succinogenes* readily degrades crystalline cellulose, but Latham et al. (21) concluded that this bacterium made only a small contribution to total cellulose digestion. Later work, however, indicated that *F. succinogenes* was present at significant numbers (20, 33), and rRNA analyses indicated that it accounted for as much as 7% of the microbial mass in the rumen (31).

When *F. succinogenes* was cocultured with the noncellulolytic ruminal bacterium *Selenomonas ruminantium*, the two bacteria were found at roughly equal numbers even though cellulose was the only energy source (28). On the basis of these results, it appeared that *S. ruminantium* was "fermenting prod-

ucts, presumably sugars or cellodextrins, produced from cellulose by *B. succinogenes*" (7). This hypothesis was supported by the observation that *S. ruminantium* can utilize water-soluble cellodextrins (26).

When *F. succinogenes* was incubated with long-chain cellodextrins (cellohexaose, cellopentaose, and cellotetraose), there was a transient increase in cellotriose and cellobiose (26). This accumulation of short-chain cellodextrins was ascribed to extracellular hydrolysis of longer-chain cellodextrins prior to transport and metabolism. Preliminary experiments, however, indicated that *F. succinogenes* was able to produce cellobiose and cellotriose by a completely different mechanism. Cultures that were provided with only glucose produced cellobiose, and cellobiose gave rise to cellotriose. The following experiments were designed to elucidate this alternative mechanism of cellodextrin production and assess its impact on the growth of *F. succinogenes* and other ruminal bacteria.

MATERIALS AND METHODS

Growth on soluble substrates. *F. succinogenes* S85 and *Streptococcus bovis* JB1 were grown anaerobically in a basal medium that contained (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, and a volatile fatty acid mixture (9). The carbohydrate source was autoclaved separately and added just prior to inoculation. The cultures were incubated anaerobically (39°C) in tubes (18 by 150 mm), 150-ml serum bottles, or a cellobiose-limited chemostat as previously described (35). Growth was moni-

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tored by the increase in optical density (600 nm, 1-cm cuvette). Cell-free supernatants were obtained by filtration (DAWP membrane filters, 0.65- μ m pore size; Millipore Corp., Bedford, Mass.) and stored at -15°C .

Cellulose chemostats. *F. succinogenes* was also grown in a segmented gas-liquid continuous-culture system on the medium of Scott and Dehority (30) with Sigmacell 20 microcrystalline cellulose (5 mg/ml) as previously described (34), except that clarified ruminal fluid was omitted from the medium. Samples (30 ml) were removed from the continuous-culture vessel with a hypodermic syringe, and well-mixed 3.0-ml subsamples were immediately vacuum filtered through 25-mm-diameter, 3- μ m-pore-size polycarbonate membranes (Poretics, Livermore, Calif.). Filtrates containing nonadherent cells were harvested in a microcentrifuge (12,000 \times g, 5 min) and washed twice with 0.9% (wt/vol) NaCl, and the final pellets were frozen. Parallel subsamples, not subjected to filtration, were harvested by centrifugation and washed to recover the total-cell pellet (adherent plus nonadherent cells).

The fraction of nonadherent cells undergoing cell division was determined by microscopy. A drop of culture filtrate (prepared as described above) was placed on a glass microscope slide and stained with crystal violet, and the resulting wet mount was examined at $\times 1,600$ magnification with a Carl Zeiss Axioskop microscope. Microscope fields were photographed with Kodak Tri-X Pan film, and cell division was scored if pinching or invagination was observed in incipient daughter cells (see Fig. 5b). Five to seven fields of the 141 to 220 cells per field photographed at different times during steady state were used for calculations. Microscopic examination also verified that no cellulose particles (or adherent cells) had passed through the 3- μ m-pore-size filter membranes.

Washed cells. Batch cultures that had been grown on glucose or cellobiose were harvested by centrifugation (2,300 \times g, 15°C , 10 min) and washed anaerobically in a salts medium (basal medium lacking yeast extract, Trypticase, NH_4SO_4 , and volatile fatty acids). The cells were resuspended in the simplified medium (approximately 160 μ g of protein per ml), and either glucose or cellobiose (45 mM hexose equivalent) was added. No growth was observed during any phase of the 24-h incubation period. Cell-free supernatants (see above) were stored at -15°C .

Cell extracts. Exponentially growing cells (approximately 640 μ g of protein per ml) were harvested by centrifugation (2,300 \times g, 5°C , 10 min) and washed anaerobically with 50 mM triethanolamine-5 mM MgCl_2 buffer (pH 7.5). Cell pellets were resuspended in 5 ml of buffer and disrupted by sonication (Branson Sonifier 200, microtip, maximum output, 50% duty cycle, 10 min, 0°C) in an anaerobic glove box. Sonicated extracts were centrifuged (15,000 \times g, 5°C , 15 min) to remove cell debris and stored at 4°C until used.

Enzymatic assays. Cellobiose and cellotriose phosphorylase activities were estimated from the appearance of glucose 1-phosphate by a phosphoglucomutase-glucose 6-phosphate dehydrogenase NADP-linked assay (50 mM triethanolamine-5 mM MgCl_2 buffer [pH 7.5], 5 μ mol of cellobiose or cellotriose, 0.8 μ mol of NADP, 8 U of phosphoglucomutase, 12 U of glucose 6-phosphate dehydrogenase, and 100 μ mol of potassium phosphate per ml). Glucose release from cellobiose was assayed by a hexokinase-glucose 6-phosphate dehydrogenase NADP-linked assay (same assay conditions as for phosphoglucomutase with 5 μ mol of ATP and 27 U of hexokinase per ml but no phosphoglucomutase).

Thin-layer chromatography. Cell extracts were incubated with cellobiose and/or potassium phosphate in sterile triethanolamine buffer at 39°C for as long as 10 h. Samples (1 ml) were treated with 0.5 ml of 12% perchloric acid and centrifuged (33,000 \times g, 22°C , 2 min) to remove cell protein. Supernatants were neutralized with 0.5 ml of KOH- KHCO_3 (1 M each) and recentrifuged to remove perchlorate. Extracts were stored at -15°C .

Samples and standards (cellobiose, glucose, or glucose 1-phosphate, 1 mM each) were spotted (2.5 μ l, four times) on a thin-layer silica gel plates (Silica Gel 60; Kieselguhr F254 plate [20 by 20 cm]), and the sugars were separated with a propanol-ethylacetate-water (7:1:4) mixture as previously described (29). Once the plates had dried, the silica gel was sprayed with anisaldehyde-ethanol-sulfuric acid-acetic acid (5:45:5:1, vol/vol; prepared fresh daily) as previously described (18). The plates were developed at 105°C , and the detection limit of glucose 1-phosphate was 10 nmol.

Cellodextrin analyses. Cell-free samples (5 ml) were mixed with 1 g of Dowex-50 W (50X4-100; Sigma Chemical Co., St. Louis, Mo.) and vortexed for 1 min. After the resin settled, 4 ml of the acidified sample was transferred to another tube. This supernatant was neutralized with 1.5 g of Dowex-2 (2X8-100; Sigma) and vortexed for 1 min. The final pH was between 6.5 and 7.0, and salt-free supernatant was removed and stored at -15°C . The cellodextrins were analyzed by high-pressure liquid chromatography (HPLC) using a Bio-Rad 42A carbohydrate column and refractive index as a detection system (26).

Other analyses. Cellular nitrogen was determined by using a Carlo Erba NA1500 combustion-type nitrogen analyzer as previously described (34). Cellulose was determined gravimetrically (34). Cellular protein was assayed by the method of Lowry et al. (23). Cellular polysaccharide was assayed by an anthrone procedure (4). Volatile fatty acids and succinate were measured by HPLC (11).

RESULTS

Cell growth and sugar utilization. *F. succinogenes* S85 grew slowly when the external glucose concentration was 5 mM, but

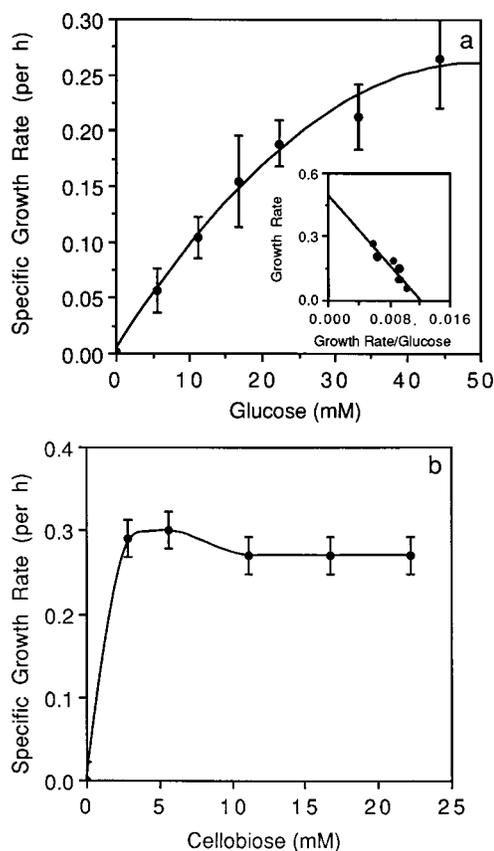


FIG. 1. The effect of glucose (a) or cellobiose (b) on the maximum specific growth rate of *F. succinogenes* in batch culture. An Eadie-Hofstee plot (velocity per substrate versus velocity) is shown as an inset in panel a.

there was a fivefold increase in the maximum specific growth rate if the concentration of glucose was increased to 45 mM (Fig. 1a). Based on the Eadie-Hofstee plot (Fig. 1, inset), the affinity constant (K_s) for glucose was greater than 10 mM. *F. succinogenes* S85 grew rapidly on cellobiose even if the concentration was as low as 2.6 mM, and there was no increase in maximum specific growth rate when cellobiose was increased from 2.6 to 22 mM (Fig. 1b). Both sugars yielded similar fermentation end products (succinate and acetate). When the sugar concentration was low, the final optical density was greater for cellobiose than for glucose, but little difference in final optical density was observed if the sugars were present at 45 mM hexose equivalent.

F. succinogenes batch cultures that were incubated with 45 mM glucose converted some of the glucose to cellobiose (Fig. 2a), and this extracellular cellobiose did not decrease until one-third of the glucose had been utilized. When the initial glucose concentration was less than 35 mM, cellobiose accumulation was not detected (Fig. 2b). Cultures that were provided with as little as 2.5 mM cellobiose produced extracellular cellotriose, and cellobiose accumulation was once again a transient phenomenon (Fig. 2a). Growing cultures never produced cellodextrins larger than cellotriose.

Cellodextrin production by washed cells. Cells that were washed anaerobically and reincubated in medium lacking ammonia did not grow, and the optical density decreased 80% within 4 h of the incubation period. Despite the rapid cell lysis, more than 75% of the cellobiose was utilized (approximately

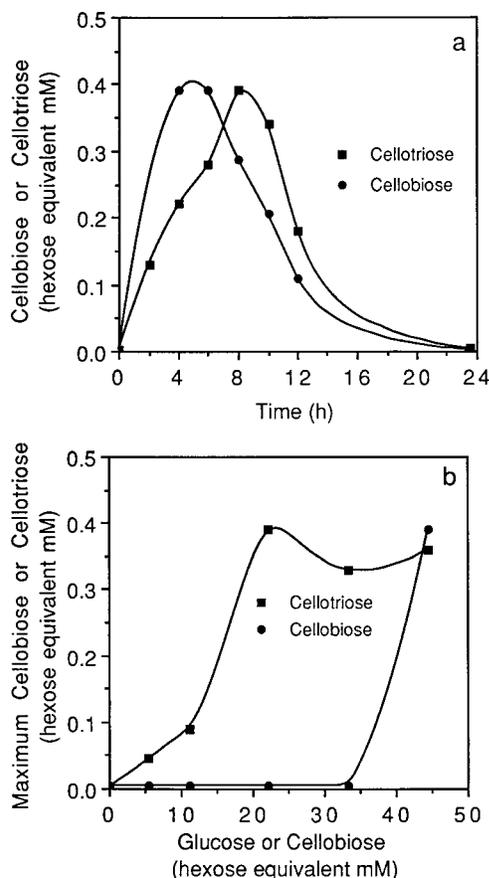


FIG. 2. (a) Accumulation of extracellular cellobiose or cellotriose when *F. succinogenes* batch cultures were provided with either 45 or 22.5 mM glucose or cellobiose, respectively; (b) relationship between the maximal accumulation of cellobiose or cellotriose and the amount of glucose or cellobiose provided, respectively.

30 mM hexose equivalent), and approximately two-thirds of this cellobiose was converted to cellotriose and longer-chain cellodextrins (Fig. 3a). The ratio of cellodextrins to cellodextrins with one more glucose unit [$n/(n+1)$] was initially greater than 6, but this ratio eventually declined to values between 3 and 5 (Fig. 3b). To verify that the products were cellodextrins, samples were incubated with a commercial amyloglucosidase. The amyloglucosidase could hydrolyze a maltodextrin mix, but it did not hydrolyze the cellodextrins in the samples (data not shown).

Cell extracts. On the basis of an enzyme assay employing phosphoglucomutase and glucose 6-phosphate dehydrogenase, glucose 1-phosphate was an end product of cellobiose or cellotriose catabolism. Little activity was noted until P_i was added to the assay (Fig. 4), and glucose 1-phosphate was not detected if glucose or no cellodextrin was added. Cellobiose phosphorylase activity was 20-fold less than the cellobiose consumption rate of exponentially growing cells (4 versus 76 nmol/mg of protein per min), but the phosphoglucomutase reaction is a reversible process that is close to equilibrium. When the glucose was removed with hexokinase and glucose 6-phosphate dehydrogenase, the rate of cellobiose degradation of the cell extracts was greater than the cellobiose consumption rate (200 versus 76 nmol/mg of protein per min). Because the ATP contained a significant amount of P_i , the latter assay was not stimulated by additional phosphate. When cell extracts were

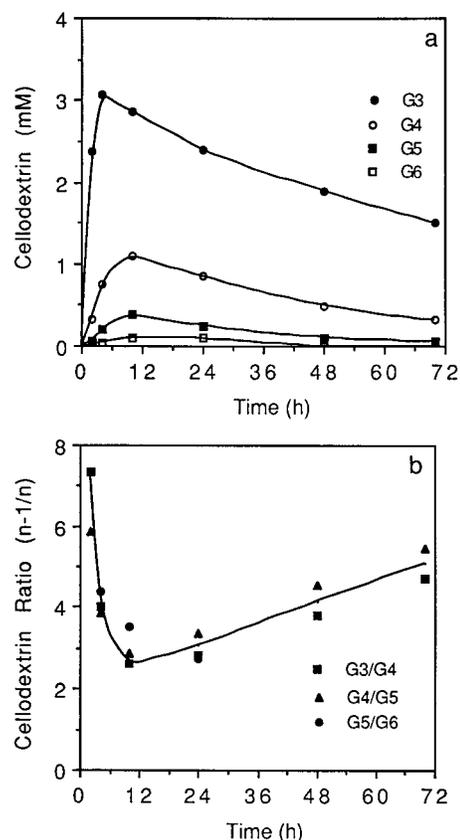


FIG. 3. (a) Accumulation of extracellular cellodextrins (as indicated by the number of glucose residues [G]) when washed cells of *F. succinogenes* were provided with 22.5 mM cellobiose; (b) ratio of extracellular cellodextrins to cellodextrins with one or more glucose unit [$n/(n+1)$].

incubated in the absence of cellobiose, no glucose 1-phosphate could be detected on thin-layer chromatography plates. Glucose 1-phosphate was only detected if phosphate was added to the cell extracts, and the intensity of the glucose 1-phosphate spot increased with time.

Continuous cultures. When *F. succinogenes* was grown in a cellobiose-limited chemostat, more than 0.5 mM cellobiose

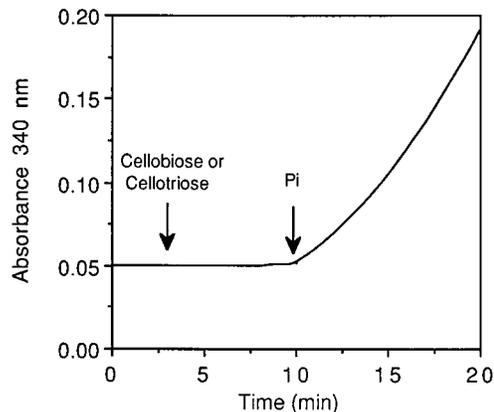


FIG. 4. The effect of 50 mM potassium phosphate addition (P_i) on the ability of *F. succinogenes* crude extract to convert either cellobiose or cellotriose to glucose 1-phosphate.

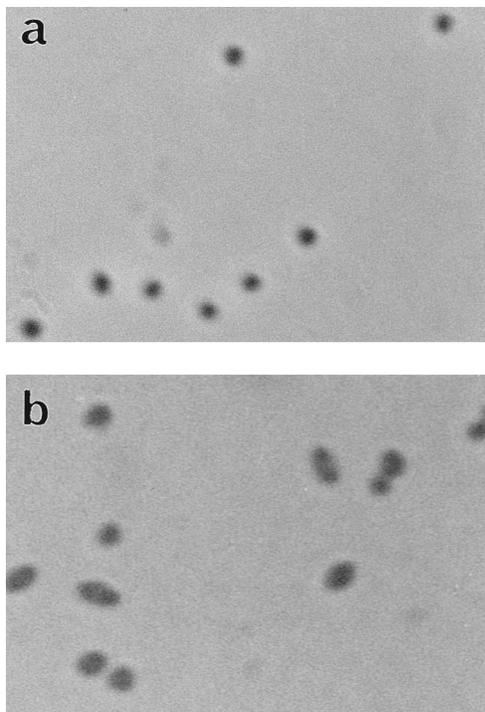


FIG. 5. Phase-contrast micrographs of stationary-phase *F. succinogenes* cells from a cellobiose-grown batch culture (a) and nonadherent *F. succinogenes* cells from a cellulose-limited continuous culture (b) (dilution rate, 0.07 h^{-1}). In both panels, 3 mm is equal to $1 \mu\text{m}$.

was detected at dilution rates greater than 0.25 h^{-1} . The cellobiose-limited continuous cultures also had some extracellular cellotriose, and the ratio of cellotriose to cellobiose was approximately 1 to 4. Cellulose-limited chemostats of *F. succinogenes* always left some undigested cellulose, and this residual cellulose was completely covered with cells. Cellodextrins could never be detected, but these cultures had a large number of unattached cells (Fig. 5b). As the dilution rate and amount of undigested cellulose declined (Fig. 6a), the percentage of nonadherent cells increased (Fig. 6b). Cultures that had depleted their cellobiose did not have any dividing cells (Fig. 5a), but the nonadherent cells of the cellulose-limited continuous cultures were observed in all stages of cell division (Fig. 5b). The percentage of nonadherent cells undergoing cell division was greatest at rapid dilution rates (Fig. 6b). The nonadherent cells had a large pool of polysaccharide reserve (polysaccharide-to-protein ratio of at least 0.4 mg/mg). When the nonadherent cells were removed from the continuous-culture device and incubated in the absence of cellulose for 8 h, the ratio of polysaccharide to protein decreased from 0.4 to 0.1 mg/mg .

Cocultures. When *S. bovis* and *F. succinogenes* were cocultured with cellulose as the only energy source, both cell types (ovoid cells and rods, respectively) persisted for at least 20 transfers. On the basis of microscopic examinations and cell morphology, it appeared that *S. bovis* accounted for approximately 20% of the coculture. Cellodextrins could not be detected in the cellulose preparation ($<0.1 \text{ mM}$ hexose equivalent), and *S. bovis* was not able to grow in the absence of *F. succinogenes*.

DISCUSSION

Scheifinger and Wolin (28) cultivated *S. ruminantium* with *F. succinogenes* with cellulose as the only added energy source,

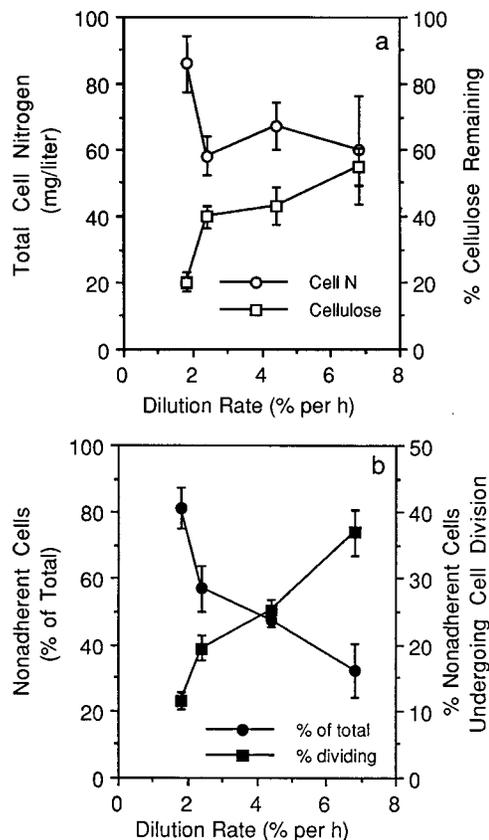


FIG. 6. (a) Growth of *F. succinogenes* in cellulose-limited continuous cultures and the amount of cell nitrogen or cellulose remaining; (b) percentage of nonadherent cells and the percentage of nonadherent cells undergoing cell division.

but this example of cellulose cross-feeding is complicated by the fact that the HD₄ strain of *S. ruminantium* produces an endo-1,4- β -glucanase (carboxymethyl cellulase [27]) and utilizes succinate, an end product of *F. succinogenes*. *S. ruminantium* cannot utilize succinate as a sole energy source for growth (28), but succinate decarboxylation is another potential mechanism of energy derivation (10). *S. bovis* can utilize short-chain cellodextrins (26) but cannot produce β -glucanases or utilize succinate. Because *S. bovis* made up a significant fraction of the cells in the cellulose coculture and the coculture persisted for many transfers, it appeared that both organisms were indeed benefitting from cellulose degradation.

On the basis of the idea that extracellular soluble saccharides would be the initial products of the cellulose degradation, Wolin (36) hypothesized that both noncellulolytic and cellulolytic species would compete for these "cellulose fragments." Kudo et al. (19) noted a commensal interaction between a spirochete and *F. succinogenes* and suggested that the noncellulolytic bacteria needed to adhere and "position themselves favorably in relation to the availability of cellulose-derived nutrients." This hypothesis of cellulose cross-feeding, however, did not consider the tight attachment of *F. succinogenes* to cellulose. Because the outer membrane of *F. succinogenes* "completely conforms to the contours of the substrate" (12) and the outer surfaces of the cell are "without external, fibrous coat materials, in most cases" (1), the direct loss of sugars from the site of cellulose digestion is difficult to envision.

Forsberg et al. (12) concurred with the idea that *F. succinogenes* and other polysaccharide-degrading bacteria could pro-

vide mono- and disaccharides to organisms lacking cellulases and hemicellulases, but they proposed a mechanism of cross-feeding that was based on subcellular cellulases. When *F. succinogenes* cultures enter the stationary phase, membrane vesicles (blebs) are often observed, and electron microscopy indicated that there might be regions of cellulose digestion adjacent to the blebs (12). Halliwell and Bryant (15), however, concluded that cellulose digestion by the cell-free enzymes was probably insignificant, and the electron microscopy studies of Akin (1) suggested that cell attachment was an obligate requirement for cellulose digestion.

Forsberg et al. (12) noted that *F. succinogenes* cultures had a significant number of cells that were not attached to cellulose, but the metabolic state of these nonadherent cells was not defined. Because the fluid phase of cellulose-limited continuous cultures had a large number of dividing cells, it appeared that nonadherent cells were utilizing cellobioses as an energy source for growth. This assumption was supported by a number of observations: (i) the polysaccharide-to-protein ratio of the nonadherent cells was five times greater than that of starved cells (35), (ii) the polysaccharide reserves of nonadherent cells decreased when no external carbohydrate was available, (iii) polysaccharide reserves alone cannot serve as an energy source for growth (35), and (iv) true stationary-phase cultures had few, if any, dividing cells.

Cellobioses could not be detected in the cellulose-limited chemostats, but the dilution (growth) rates of these cultures were very low. Based on an affinity constant (K_S) of 400 μM (35), a maximum specific growth rate (μ_{max}) of 0.35 h^{-1} , and the standard Monod relationship [$\mu = (\mu_{\text{max}} \cdot S)/(K_S + S)$], the external concentration of cellobiose needed to maintain a growth rate (μ) of 0.015 h^{-1} would be 15 μM . Since the detection limit of our HPLC analyses for an individual cellobiose was at least 25 μM glucose equivalent, it is not surprising that cellobioses could not be detected in the low-dilution-rate, cellulose-limited chemostats.

Many cellulolytic bacteria have cellobiose phosphorylases that conserve the free energy of the glycosidic bond as glucose 1-phosphate (25). Groleau and Forsberg (14) indicated that *F. succinogenes* did not have a phosphorylase, but the silver nitrate-acetone system that they used is not a sensitive method for detecting phosphorylated sugars (22). Their "cellobiase" activity was stimulated by sodium phosphate addition, but this effect was interpreted as relief of a Tris buffer inhibition rather than a phosphate requirement per se. Our work indicated that *F. succinogenes* had cellobiose and cellobiosyl phosphorylase activities, and these reactions operated in a reversible fashion to either degrade or synthesize cellobioses.

The equilibrium constant (K_{eq}) of cellobiose phosphorylase is 4 (2), and the cellobiosyl production of *F. succinogenes* mimicked this 4-to-1 relationship. Washed cells produced cellobioses with as many as six glucose units, and the ratio of cellobioses to cellobioses with one greater glucose unit [$n/(n + 1)$] was approximately 4. Cellobiose-limited chemostats left both cellobiose and cellobiosyl, and the ratio of cellobiose to cellobiosyl was 4. *S. bovis* persisted with *F. succinogenes* in cellulose batch cultures, and the ratio of *F. succinogenes* to *S. bovis* was approximately 4. On the basis of these results, the cellobiosyl production of *F. succinogenes* seems to be an equilibrium (mass action) function and not just an artifact of energy-rich cultural conditions.

Further work is needed to more fully evaluate the energetics of cellobiosyl efflux, but it should be noted that phosphorylases (2) and sodium symport reactions (16) are both reversible. Franklund and Glass (13) demonstrated that *F. succinogenes* had a sodium symport mechanism for glucose transport,

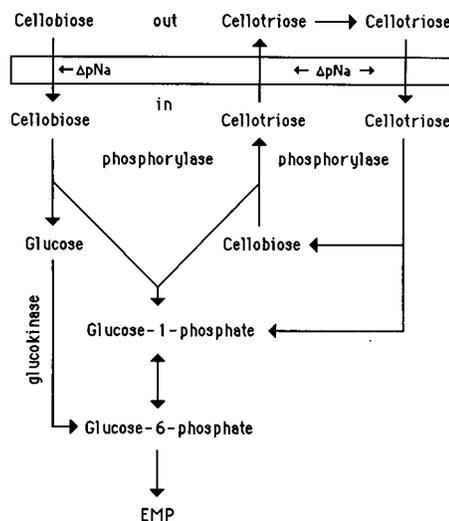


FIG. 7. Hypothetical schemes of cellobiosyl production and utilization by *F. succinogenes* cultures fermenting cellobiose. Transport and efflux are linked to sodium motive force ($\Delta p\text{Na}$), and final metabolism proceeds by the Embden-Meyerhof-Parnas pathway (EMP).

and later work indicated that it had a separate sodium-dependent mechanism for cellobiose (24). The mechanism of cellobiosyl translocation is not known, but *F. succinogenes* was never able to ferment cellulose in the absence of sodium (data not shown). If cellobiosyl efflux recreated the same sodium motive force as the amount needed for sodium-dependent cellobiosyl uptake, the cycle of cellobiosyl uptake and efflux would not demand any additional energy (Fig. 7). Such a scheme, however, does not consider the impact on individual cells. If the cell that secreted the cellobiosyl was not the one that recovered it, there would be a transfer of carbon and energy. Because a variety of noncellulolytic ruminal bacteria are also able to utilize cellobioses (26), the growth potential of *F. succinogenes* in a mixed-culture environment like the rumen could be compromised.

Microorganisms in natural environments are sometimes involved in commensal or mutualistic relationships, but in such interactions each organism usually has a well-defined primary niche. For instance, *F. succinogenes*, a carbohydrate-fermenting bacterium, requires branched-chain volatile fatty acids (5) that are provided by amino acid-degrading ruminal bacteria (3). The phenomenon of cellobiosyl efflux represents a more complicated interaction. Adherent cells share carbon and energy with nonadherent cells. At first sight, this interaction looks like an altruistic event that benefits the entire community, but this argument is probably nothing more than a teleological justification. Since the cell losing carbon and energy via cellobiosyl efflux would receive no immediate advantage, there is no apparent basis for natural (genetic) selection. In reality, the metabolic scheme of *F. succinogenes* probably reflects a compromise between the energetic advantage of the reversible phosphorylase reaction and the potential loss of carbon and energy as cellobioses.

The impact of cellobiosyl efflux on cellulose digestion is not readily apparent. When Stanton and Canale-Parola (32) cocultured a noncellulolytic treponema strain with *F. succinogenes*, no increase in either the amount or rate of cellulose digestion was noted. Kudo et al. (19) indicated that a coculture of *F. succinogenes* and a treponema fermented barley straw faster than a pure culture, but the rate of cellulose digestion per se

was not measured. Because the cellulose-limited chemostats always had an excess of nonadherent cells, it appeared that surface area was a more important determinant of cellulose digestion than celldextrin efflux and the number of nonadherent bacteria.

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