INFECTION AND IMMUNITY, Dec. 1991, p. 4547–4554
0019-9567/91/124547-08$02.00/0

Vol. 59, No. 12

Sugar Metabolism by Fusobacteria: Regulation of Transport, Phosphorylation, and Polymer Formation by *Fusobacterium mortiferum* ATCC 25557

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Received 13 May 1991/Accepted 12 September 1991

Strains of eight *Fusobacterium* species differed in the ability to use sugars as energy sources for growth. For *Fusobacterium russii* ATCC 25533, *F. gonidiaformans* ATCC 25563, and *F. nucleatum* ATCC 10953 (except for frutose), growth was marginal to poor on all of the sugars tested. Other species displayed reasonable growth on glucose, fructose, mannose, and galactose, and two strains of *F. mortiferum* (ATCC 25557 and ATCC 9817) grew well on six of the sugars tested, including sucrose and maltose. Glucose transport by resting cells of most of the species was dependent upon (or markedly stimulated by) the presence of a fermentable amino acid. By contrast, *F. mortiferum* cells rapidly accumulated glucose and other sugars in the absence of amino acids. Although these cells were constitutive for glucose uptake, accumulation of other sugars was specifically induced by growth of *F. mortiferum* on the appropriate sugar. Spectrophotometric analyses and in situ staining of anionic polyacrylamide gels showed that glucose and fructose (mannose) are phosphorylated by separate ATP-dependent kinases. Fructokinase was stable in air at 4°C, but under these conditions, >70% of the glucokinase activity was lost. After overnight dialysis of the extract, no glucokinase activity was detectable; however, 65% of the initial enzyme activity was retained by inclusion of 1 mM dithiothreitol in the dialysis buffer. Thin-section electron microscopy showed that cells of *F. mortiferum* produced various amounts of intracellular glycogen during growth on the following sugars (in decreasing order of formation): galactose > sucrose > glucose > mannose > fructose. Mechanisms for sugar transport regulation, phosphorylation, and polymer synthesis by *F. mortiferum* cells are proposed.

Fusobacteria are morphologically diverse anaerobic gram-negative rods (5, 20, 21) found frequently (often in mixed culture; 6, 12, 19, 39) in a variety of human and animal infections (12, 19, 28, 35). The prevalence and increased numbers of particular species (e.g., *Fusobacterium nucleatum*, *F. alocis*, and *F. sulci* [8]) in the subgingival areas of patients with gingivitis and periodontitis (18, 26–28) suggest that fusobacteria are contributory agents in the etiology of these oral diseases (28, 36).

Fusobacteria generally grow well in media containing Trypticase, peptone, or yeast extract (1, 11, 15–17), and the evidence indicates that amino acid catabolism (2, 3, 7, 23, 25, 30) provides the energy (and requisite nitrogen) for growth of many, if not all, species. With respect to sugar metabolism, the fusobacteria are often described as asaccharolytic or weakly fermentative (20, 21, 40). However, there is evidence of limited utilization of glucose and certain other sugars by members of this group, but the data are fragmentary and sometimes confusing. For example, 40 years ago Jackins and Barker (23) described the stimulatory effect of glucose on *F. nucleatum* growth but there was little utilization of the sugar from the medium. Later, Coles (9) and Loesche and Gibbons (25) described the limited metabolism of glucose by *F. nucleatum* but questioned the lack of correlation between sugar disappearance and expected recovery of fermentation products. Recent experiments performed with nongrowing cells in our laboratory (30–33) have provided answers to some of the perplexing aspects of glucose metabolism in *F. nucleatum* by the findings that (i) accumulation of glucose (and galactose) by the cells is dependent upon energy generated by amino acid fermentation, (ii) intracellular sugar phosphates are rapidly transformed into a glycogenlike polymer, and (iii) fermentation of amino acid prevents catabolism of endogenous polymer reserves. These observations have largely been confirmed for growing *F. nucleatum* cells maintained in continuous culture (34).

From the previous observations with *F. nucleatum*, it seemed appropriate to ask whether accumulation of sugars by other species of asaccharolytic fusobacteria would be similarly regulated by amino acid fermentations. We have addressed this question, and our findings are presented in this communication. Compared with other species, *F. mortiferum* exhibited the unexpected ability to utilize a variety of sugars as energy sources for growth. These aspects of sugar metabolism by *F. mortiferum* have been studied in detail.

MATERIALS AND METHODS

Bacterial cultures. All species of fusobacteria were obtained from the American Type Culture Collection, Rockville, Md. Cultures were maintained in a modified thioglycolate medium described previously (30).

Growth studies. Organisms were grown anaerobically (Gas Pak; BBL Microbiology Systems, Cockeysville, Md.) at 37°C in screw-cap tubes containing 10 ml of modified Todd-Hewitt broth (MTB; 30). When required, appropriate sugars or amino acids were included in this medium at a final concentration of 0.25% (wt/vol). Growth was monitored turbidimetrically in a Klett-Summerson colorimeter

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TABLE 1. Growth of fusobacteria on MTB supplemented with various sugars as potential energy sources[a]

Species	ATCC no.	Growth with the following sugar[b] added to MTB:					
		Glucose	Fructose	Galactose	Mannose	Sucrose	Maltose
F. nucleatum	10953	+	++	+	−	−	−
F. varium	8501	+++	+++	++	+++	−	−
F. russii	25533	−	−	−	−	−	−
F. perfoetens	29250	++	++	++	++	++	−
F. necrophorum	25286	+++	++	++	−	−	+++
F. necrogenes	25556	−	±	++	±	−	−
F. gonidiaformans	25563	−	−	−	−	−	−
F. mortiferum	25557	+++	+++	+++	+++	+++	+++
F. mortiferum	9817	+++	+++	+++	+++	+++	+++

[a] Growth was determined as described in Materials and Methods, and extent of growth is represented by the following symbols: −, no growth (i.e., no increase in Klett units above the control [inoculated but unsupplemented MTB]); ±, marginal growth; +, 30 to 50 Klett units; ++, 50 to 100 Klett units; +++, maximum growth between 100 and 165 Klett units.

[b] Sugars were added to MTB to a final concentration of 0.25% (wt/vol).

equipped with a red filter. Calibration curves relating Klett units and cell protein were prepared for each species.

Growth of *F. mortiferum* for transport, electron microscopy, and enzyme studies. Batch cultures of *F. mortiferum* ATCC 25557 were grown anaerobically to the stationary phase in 200 ml of MTB supplemented with 0.25% (wt/vol) of the appropriate sugar or amino acid as the energy source. Cells were collected from the culture by centrifugation at 5,000 × *g* for 20 min at 4°C. To maintain anaerobic conditions, the tubes were flushed with a mixture of anaerobic gas (5% CO_2, 5% H_2, 90% N_2). The cell pellets were immediately suspended in the required volume of anaerobically prepared buffer (referred to as Coles' buffer [9, 30]) containing 50 mM potassium phosphate (pH 7), 0.7 mM $MgCl_2$, 0.1 mM AMP, and 0.1 mM NAD^+. The cell suspensions were maintained at 0°C until required.

Preparation of cell extracts. Washed cells of *F. mortiferum* obtained from a 200-ml culture volume (approximately 1 g [wet weight] of cells) were suspended in an equal volume of Coles' buffer. The cells were disrupted (at 0°C under anaerobic gas) with the Microtip probe of a Branson Sonifier. After three 15-s periods of sonication with intermittent cooling, intact cells and cell debris were removed by centrifugation (27,000 × *g* for 30 min at 4°C). The clarified supernatant fluid was removed for enzymatic analyses. Kinase activity (phosphorylation of glucose, fructose, and mannose) was determined spectrophotometrically (at 340 nm) by using an $NADP^+$-linked coupled enzyme assay containing, in 1 ml, 0.1 M potassium phosphate buffer (pH 7.0), 10 mM ATP, 10 mM $MgCl_2$, 1 mM $NADP^+$, 10 mM sugar, 5 U of glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 5 U of phosphoglucose isomerase (EC 5.3.1.9), and cell extract (usually 20 μl [~500 μg of protein]). For determination of mannose phosphorylation, 10 U of phosphomannose isomerase (EC 5.3.1.8) was included in the assay. Kinase activities are expressed as nanomoles of sugar phosphorylated per milligram of protein per minute at room temperature (ca. 22°C). Protein was determined by modification of the Lowry procedure as described in a previous communication (33).

Anionic polyacrylamide gel electrophoresis (PAGE) and kinase activity staining. The procedure of Davis (10) was used for anionic PAGE, and all experiments were performed in an SE 600 vertical slab gel electrophoresis unit (Hoefer Scientific Instruments). After completion of electrophoresis, the gel was cut into slices and one slice was stained for protein with Coomassie G-250. The remaining slices were incubated for 20 min in 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.5) containing 5 mM $MgCl_2$. Kinase activities in gel slices were then detected by the method of Gabriel (14) with glucose, fructose, and mannose as substrates.

Sugar transport by resting cells. The procedure used to monitor sugar transport by washed (resting) cells of *F. mortiferum* was essentially that described earlier for *F. nucleatum* (30, 33). Sugar transport experiments were performed at 37°C (usually anaerobically) in an assay (5 ml) containing 0.5 to 1.5 mg of cell protein per ml in Coles' buffer, 0.5 mM ^{14}C-labeled sugar (specific activity, 0.2 μCi/μmol), and, when required, the appropriate amino acid at 8 mM. Initial rates of sugar uptake (nanomoles of sugar accumulated per milligram of cell protein per minute) were calculated from tangents drawn to the progress curves.

Utilization of sugars by resting cells. Washed cells (equivalent to 2 to 3 mg of total cell protein) were suspended in 5 ml of Coles' buffer supplemented with 2 mM glucose or fructose. At intervals, 0.5 ml of the suspension was withdrawn by syringe and cells were removed by membrane filtration (Millex-GS membrane; 0.22-μm pore size; Millipore Corp.). Filtrates were collected, and residual glucose was determined by Glucose Analysis Kit 115 (Sigma Chemical Co., St. Louis, Mo.). Fructose was similarly assayed after addition of phosphoglucose isomerase to the system.

Electron microscopy. The conditions used for preparation, fixing, embedding, and sectioning of *F. mortiferum* cells have been described in previous communications (30, 33).

Reagents. Radiolabeled sugars were obtained from Amersham and Dupont NEN Research Products (Boston, Mass.). Amyloglucosidase (1,4-α-D-glucan glucohydrolase) was purchased from Boehringer Mannheim Biochemicals, and sugars were from Pfanstiehl Laboratories, Inc., Waukegan, Ill. Glucose reagent kits and other enzymes and biochemicals were supplied by Sigma.

RESULTS

Growth of fusobacteria. As a first step in our investigation, the abilities of various fusobacteria to grow in basal medium supplement with different sugars were examined (Table 1). In the basal unsupplemented medium (i.e., no sugar added), growth of all species was barely detectable. Inclusion of sugars in this medium permitted growth of some species, but little to no growth was detectable with others, e.g., *F. russii*, *F. gonidiaformans*, and *F. necrogenes*. Fructose permitted

TABLE 2. Amino acid dependencies of various species of fusobacteria for glucose uptake[a]

Species	ATCC no.	Amino acid in transport assay[b]	
		Absent	Present
F. nucleatum	10953	<0.1	8.5
F. varium	8501	—[c]	5.6
F. russii	25533	—	6.3
F. perfoetans	29250	—	5.0
F. necrophorum	25286	1.9	6.9
F. necrogenes	25556	—	8.5
F. gonidiaformans	25563	0.4	1.4
F. mortiferum	25557	5.5	9.5

[a] Organisms were grown in MTB supplemented with 0.25% (wt/vol) glutamate (sodium salt), except *F. necrogenes* and *F. mortiferum*, which received 0.25% (wt/vol) serine as the energy source.

[b] Conditions for sugar transport are described in Materials and Methods. When required, either 8 mM sodium glutamate or 8 mM serine was included in the transport assay. Values are expressed as nanomoles of glucose accumulated per milligram of cell protein per minute.

[c] —, no detectable sugar accumulation by the cells.

good growth of *F. nucleatum* (33), but growth at the expense of glucose or galactose was marginal to poor. Glucose, fructose, and galactose permitted fair to good growth of *F. varium*, *F. perfoetens*, and *F. necrophorum*, but growth of these species was variable on other sugars. Significantly, and in contrast to other species, both strains of *F. mortiferum*, ATCC 9817 and ATCC 25557, grew well on all of the sugars tested.

Glucose transport by fusobacteria. Previously we showed that glucose accumulation by *F. nucleatum* ATCC 10953 was dependent upon energy derived from fermentation of an amino acid such as glutamate or lysine (30). The generality of this requirement for glucose uptake by other species of fusobacteria was examined (Table 2). For these studies, all organisms were grown in the basal medium supplemented with a high concentration of a fermentable amino acid (glutamate or serine) as the energy source. The transport experiments showed that nongrowing cells of most species failed to accumulate [^{14}C]glucose unless provided with a fermentable amino acid. *F. mortiferum* (and, to a degree, *F. necrophorum*) was again the exception, and these cells accumulated the sugar in the absence of an amino acid (Table 2). The data permitted two conclusions to be drawn, i.e., that (i) a constitutive glucose transport system is present in all species or (ii) for most species, glucose accumulation is dependent upon, or markedly enhanced by, the presence of a fermentable amino acid.

Inducibility of sugar transport systems in *F. mortiferum*. The unique ability of *F. mortiferum* to grow on all sugars prompted us to study the characteristics of sugar transport in strain ATCC 25557. In preliminary studies, cells were grown in medium containing glucose or mannose and the ability of washed cells to accumulate various ^{14}C-labeled sugars was examined (Fig. 1A and B, respectively). Glucose-grown cells rapidly accumulated glucose, but other sugars were transported poorly, if at all. Cells grown previously on mannose also accumulated glucose, but importantly, these cells were also induced for rapid uptake of mannose. To investigate the inducibility of other transport systems, *F. mortiferum* cells were grown on a specific sugar and the ability of washed cells to transport a variety of other sugars was determined (Table 3). The inducibility of sugar accumulation is suggested by the fact that in most cases the initial rate of uptake

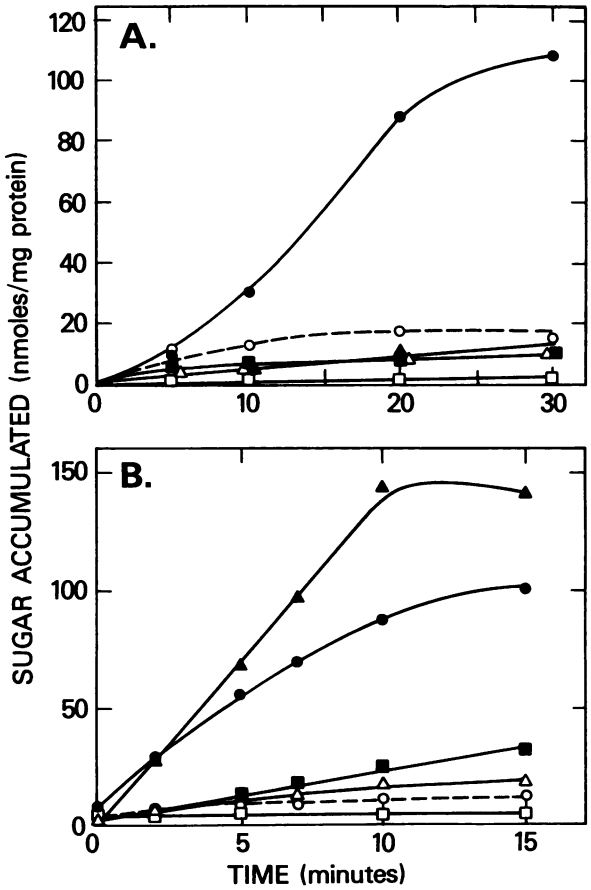

FIG. 1. Accumulation of sugars by resting cells of *F. mortiferum* grown previously on glucose (A) or mannose (B). Washed cells were suspended in buffered medium containing the appropriate ^{14}C-labeled sugars at a concentration of 0.5 mM (specific activity, 0.2 μCi/μmol). Symbols: ●, glucose; △, fructose; ○, galactose; ▲, mannose; ■, sucrose; □, maltose.

of a particular sugar was greatest when the organism had previously been grown on that sugar. The constitutivity of the glucose transport system (Fig. 1) was confirmed by the finding that amino acid (serine)-grown cells and sugar-grown organisms transported glucose at comparable rates (Table 3).

TABLE 3. Initial rates of sugar uptake by washed cells of *F. mortiferum* ATCC 25557 grown previously on either serine or a specific sugar[a]

Growth substrate	Uptake of ^{14}C-labeled sugar in transport assay[b]				
	Glucose	Fructose	Galactose	Mannose	Sucrose
Serine	5.0	1.0	1.0	1.0	2.0
Glucose	**6.0**	0.6	0.6	0.6	0.3
Fructose	3.5	**8.5**	1.0	1.0	1.5
Galactose	5.0	0.6	**11.0**	0.4	0.4
Mannose	9.6	1.1	1.0	**12.7**	2.0
Sucrose	4.7	5.3	—	—	**4.7**

[a] Cells were grown in MTB containing 0.25% (wt/vol) serine or an appropriate sugar.

[b] Values are initial rates of sugar uptake expressed as nanomoles of sugar accumulated per milligram of cell protein per minute. —, no detectable sugar uptake. Boldface values indicate initial rates of sugar uptake by *F. mortiferum* cells grown previously on that sugar.

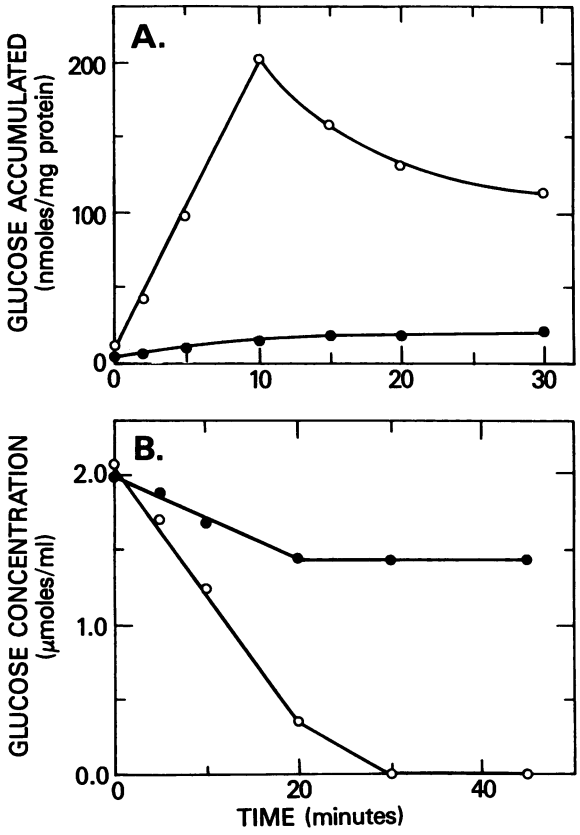

FIG. 2. Accumulation (A) and metabolism (B) of glucose by resting cells of *F. mortiferum* maintained under aerobic (●) or anaerobic (○) conditions. For panel A, washed cells grown previously on glucose were suspended in buffer containing 0.5 mM [^{14}C]glucose (specific activity, 0.2 μCi/μmol) and sugar uptake was monitored under aerobic (●) or anaerobic (○) conditions. For panel B, cells were incubated in buffered medium containing 2 mM glucose. Utilization of sugar by the cells was monitored under aerobic (●) or anaerobic (○) conditions as described in Materials and Methods.

Glucose transport and metabolism in *F. mortiferum*. Resting cells maintained under anaerobic conditions readily accumulated [^{14}C]glucose (20 nmol of sugar mg of protein^{-1} min^{-1}), but in air, the sugar uptake rate decreased by approximately 95% (Fig. 2A). Subsequent studies showed that a suspension of washed cells rapidly cleared glucose from the medium under anaerobic conditions (110 nmol of glucose metabolized mg of protein^{-1} min^{-1}). However, under aerobic conditions, the glucose utilization rate was reduced by ~70% (38 nmol of sugar metabolized mg of protein^{-1} min^{-1}) and after ~20 min of exposure to air, glucose metabolism ceased (Fig. 2B).

Phosphorylation of sugars by extracts of *F. mortiferum*. Prior to entry into the Embden-Meyerhof pathway, sugars must be phosphorylated by *F. mortiferum* either during transport (e.g., via phosphoenolpyruvate-dependent sugar: phosphotransferase [PEP-PTS] systems; 29) or by intracellular ATP-dependent kinases. Sucrose-PEP-PTS activity has been detected in permeabilized cells and by in vitro assay with preparations of disrupted cells (29a). PEP-PTS-mediated phosphorylation of other sugars, including glucose, 2-deoxy-D-glucose, mannose, and fructose, was not demonstrable. However, ATP-dependent kinase(s) activity has been found in extracts prepared from cells grown on various sugars (Table 4). Comparable rates of ATP-dependent phosphorylation of glucose, fructose, and mannose were determined in all extracts, suggestive of the constitutive nature of the phosphorylating enzyme(s). Kinase activities were maintained by storage of the extracts at 4°C under anaerobic conditions, but in the presence of air approximately 50% of the glucokinase activity was lost within 24 h (data not shown). Results from dialysis experiments (Table 5) confirmed the instability of the glucokinase and showed that this activity was partially maintained by inclusion of 1 mM dithiothreitol (DTT) in the dialysis buffer. To determine whether the three sugars were phosphorylated by one (or more) enzyme(s), electropherograms were stained for kinase activity with each sugar as the substrate (Fig. 3). The data suggested that mannose and fructose were phosphorylated by the same enzyme (i.e., a mannofructokinase [Fig. 3, lanes 3 and 4, respectively]). A separate glucokinase catalyzes glucose phosphorylation (Fig. 3, lane 5). Phosphorylation of the products of sucrose hydrolysis was detected primarily in extracts prepared from sucrose-grown cells (Table 4). This activity may be due to induction of both invertase and sucrose 6-phosphate hydrolase. The latter enzyme also catalyzes the hydrolysis of sucrose (to glucose and fructose) and sucrose-6-phosphate (to glucose-6-phosphate plus fructose). The extract of sucrose-grown cells contained high

TABLE 4. ATP-dependent phosphorylation of sugars by cell extracts of *F. mortiferum* ATCC 25557

Growth substrate[a]	Rate of sugar phosphorylation[b]			
	Glucose	Fructose	Mannose	Sucrose[c]
Serine	52.3	7.6	2.5	6.6
Glucose	68.2	6.5	2.1	3.0
Fructose	40.3	6.9	1.4	1.8
Galactose	90.8	8.6	2.0	14.7
Mannose	49.8	5.5	2.6	2.1
Sucrose	36.8	48.2	9.4	46.8

[a] The organism was grown in MTB medium supplemented with 0.25% (wt/vol) serine or the appropriate sugar.
[b] The spectrophotometric assay for sugar phosphorylation is described in Materials and Methods. Rates are expressed as nanomoles of sugar phosphorylated per milligram of protein per minute.
[c] Sucrose phosphorylation reflects ATP-dependent phosphorylation of the products (glucose and fructose) of sucrose hydrolysis by sucrose 6-phosphate hydrolase and, perhaps, invertase activities.

TABLE 5. Effect of dialysis with and without DTT on stability of ATP-dependent kinase activity in cell extracts of *F. mortiferum* ATCC 25557[a]

Sample preparation[b]	Rate of sugar phosphorylation[c]		
	Glucose	Fructose	Mannose
None (fresh cell extract)	32.2	6.5	3.4
Dialysis, DTT added	15.2	5.7	2.3
Dialysis, no DTT	<0.1	5.1	1.0

[a] The organism was grown in MTB medium containing 0.25% (wt/vol) fructose as the energy source.
[b] The freshly prepared cell extract was first assayed for kinase activities, and thereafter 2-ml samples of the extract were dialyzed overnight against 1 liter of 50 mM HEPES buffer, pH 7.5, containing 5 mM $MgCl_2$ to which 1 mM DTT was added when required.
[c] The spectrophotometric assay of sugar phosphorylation used is described in Materials and Methods. Results are expressed as nanomoles of sugar phosphorylated per milligram of protein per minute.

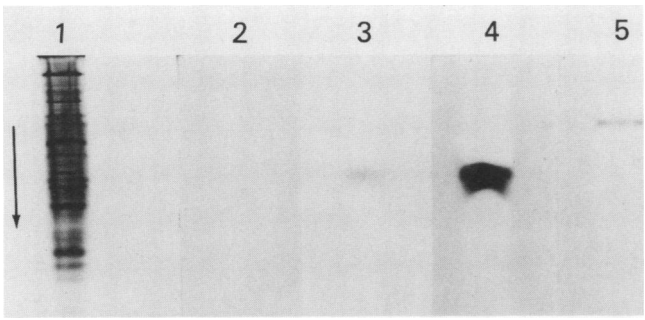

FIG. 3. Anionic PAGE and in situ staining for ATP-dependent kinase activity in an extract prepared from serine-grown *F. mortiferum* cells. Approximately 100 μg of protein was applied per lane, and after completion of electrophoresis the gel was cut into slices. One slice (lane 1) was stained for protein with Coomassie blue G-250. Other slices were incubated with appropriate sugars in the dye-enzyme coupled mixture to localize kinase activities. Lanes: 2, control (no sugar added); 3, mannose; 4, fructose; 5, glucose. The arrow indicates the direction of migration.

levels of an ATP-dependent mannofructokinase, and this enzyme has recently been purified to homogeneity (36a).

Sugar metabolism and polymer formation by *F. mortiferum*. Thin-section electron microscopy (Fig. 4) revealed granular deposits similar in size and distribution to those found previously in *F. nucleatum* (30, 33). However, the electron microscopic study showed unexpectedly that the extent of polymer deposition was dependent upon the sugar used for growth, as follows (in decreasing order): galactose > sucrose > glucose > mannose > fructose. The appearance of galactose-grown cells of *F. mortiferum* in thin sections (Fig. 4F) (where the cytoplasmic area was filled with intracellular granules) contrasts dramatically with that of those prepared from serine- or fructose-grown organisms, in which polymer deposits were barely discernible (Fig. 4A and B, respectively).

DISCUSSION

The results of our investigation may be summarized as follows. (i) Fusobacteria exhibit different abilities to utilize sugars as energy sources for growth (Table 1). When allowance is made for differences in growth conditions and medium composition, the results agree reasonably well with the tabulated information for these species in *Bergey's Manual of Systematic Bacteriology* (21) and in the Virginia Polytechnic Institute *Anaerobe Laboratory Manual* (20). (ii) For most species (as for *F. nucleatum*), glucose transport is either dependent upon or markedly enhanced by amino acid fermentation. (iii) One species—*F. mortiferum*—appears to be unique in that it does not require an amino acid for sugar transport and, significantly, has the ability to metabolize a wide variety of sugars as energy sources for growth. For these reasons, we examined the regulatory aspects of sugar transport and metabolism in *F. mortiferum* ATCC 25557 in some detail.

From the data presented in Table 3, one can reasonably conclude that the rates of accumulation of radioactivity from ^{14}C-labeled sugars by *F. mortiferum* are greatest when the cells were grown previously on that sugar. However, the data in Table 3 must be interpreted with caution, because uptake of ^{14}C label may indicate (i) induction of sugar-specific transporters, (ii) induction of sugar-specific kinases (which would accelerate catabolism of intracellular sugar), or (iii) a combination of i and ii. We cannot state unequivocally that the data represent induction of specific transport systems. However, this is highly likely, because extracts of cells grown on the various sugars (except sucrose) exhibited comparable levels of ATP-dependent kinase activities for glucose, fructose, and mannose (Table 4).

Previous investigations of sugar transport by *F. nucleatum* (30, 33) and species of the related genus *Bacteroides* (13, 22) provided no firm evidence for PEP-PTS activity in these organisms. These findings indicated that active transport systems combined with ATP-dependent kinases mediated the entry and phosphorylation of sugars in these organisms. Nonspecific hexokinase activity has been described in *Bacteroides thetaiotaomicron* (22), but for *F. mortiferum*, separate enzymes (differing in O_2 sensitivity and stabilization by DTT) catalyze the phosphorylation of glucose and fructose (mannose) prior to metabolism of these sugars via the Embden-Meyerhof pathway. In the course of this investigation (data not shown), we demonstrated (i) PEP-PTS activity in permeabilized cells, (ii) sucrose 6-phosphate hydrolase activity, and (iii) sucrose-inducible ATP-dependent mannofructokinase activity in extracts of *F. mortiferum*. The latter enzyme has been purified to homogeneity (36a). These data provide the first evidence for a sucrose operon comprising genes for sucrose-PEP-PTS, sucrose-6-phosphate hydrolysis, and fructose phosphorylation in *Fusobacterium* species.

Wahren (38), van Houte (37) and Robrish et al. (30, 33) have described polymer formation in the genus *Fusobacterium*. Similar intracellular deposits have also been reported in *Bacteroides* species (24), and the finding of an endogenous polymer in *F. mortiferum* was not unexpected. However, that the extent of polymer formation might be dependent upon the nature of the growth sugar (Fig. 4) was a result we had not anticipated and which has not previously been documented. In all cases, treatment of the extracted polymer with amyloglucosidase (4, 24) yielded [^{14}C]glucose (data not shown). Since this enzyme hydrolyzes α(1→4)- and α(1→6)-glucosidic linkages, the polymer is probably glycogen (24, 33, 37, 38). It is evident from the electron micrographs that polymer levels in cells grown on the different sugars vary dramatically, but the reasons for this are not immediately apparent. It is possible that the extent of polymer deposition is dependent upon the point of entry of the (phosphorylated) sugar into the glycolytic pathway. For example, glucose, galactose, and other sugars (which yield glucose or glucose-6-phosphate directly) may be preferentially directed toward polymer synthesis. By contrast, sugars which yield fructose or fructose-6-phosphate directly (e.g., fructose, mannose, and the fructosyl moiety of sucrose) may be selectively channeled into the Embden-Meyerhof pathway. The directional control point is not known; however, the activity of phosphohexose isomerase may regulate the flow of glycolytic intermediates in anabolic or catabolic directions (Fig. 5).

The results of our investigation show (with the exception of *F. mortiferum*) that the interdependence between amino acid fermentation and sugar transport and dissimilation described previously in *F. nucleatum* (30) pertains to fusobacteria in general. To various degrees, these anaerobic organisms derive energy from both amino acid and sugar fermentations. Fusobacteria have the ability to regulate the deposition and subsequent mobilization of endogenous sugar reserves in response to amino acid availability. These factors may contribute to the survival and persistence of these important human and animal pathogens.

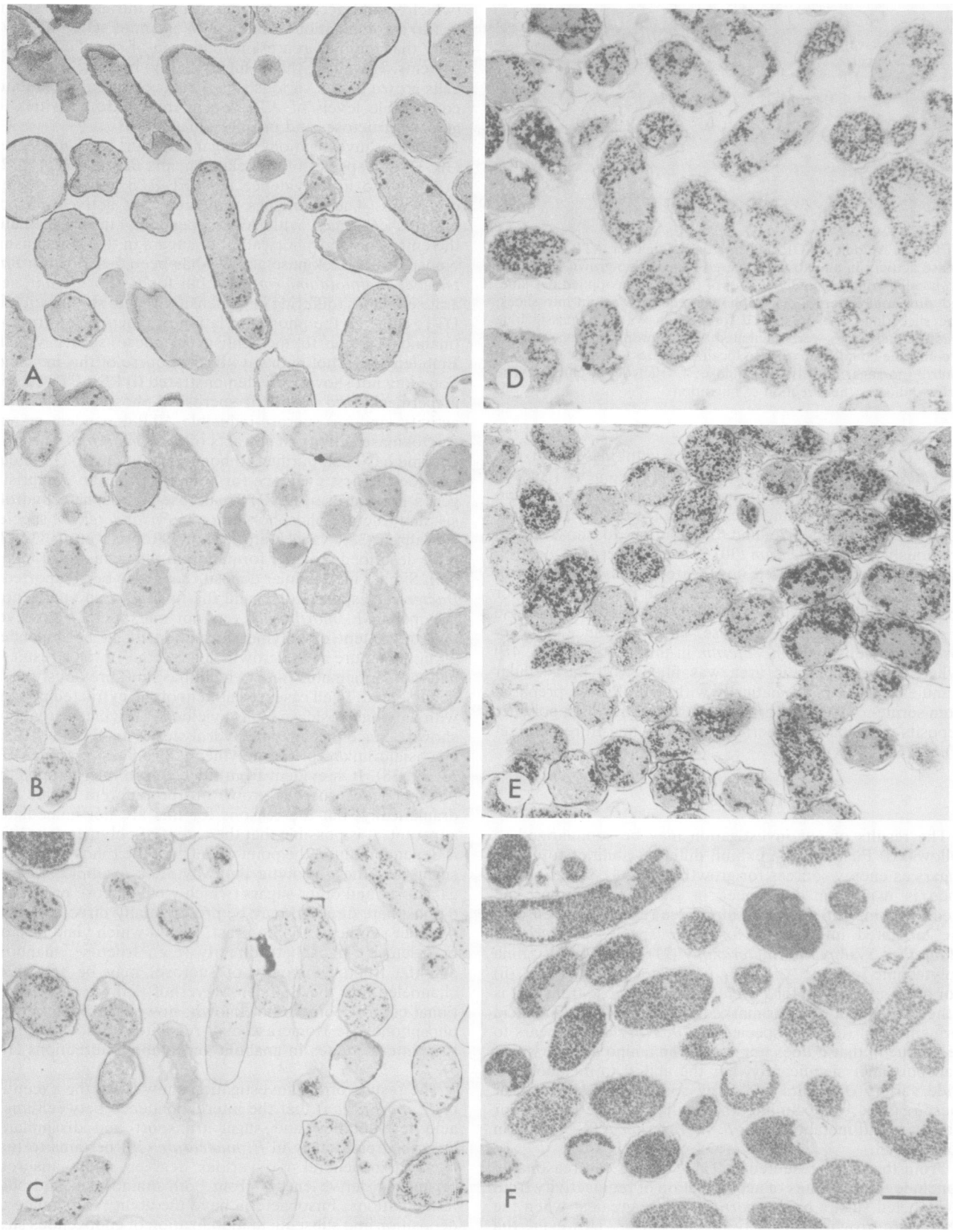

FIG. 4. Thin-section electron micrographs of *F. mortiferum* cells grown in MTB medium supplemented with an amino acid (serine) (A), fructose (B), mannose (C), glucose (D), sucrose (E), or galactose (F). Note the progressive increase from A to F in the density of intracellular glycogen granules. Magnification, ×10,000. Bar, 1 μm.

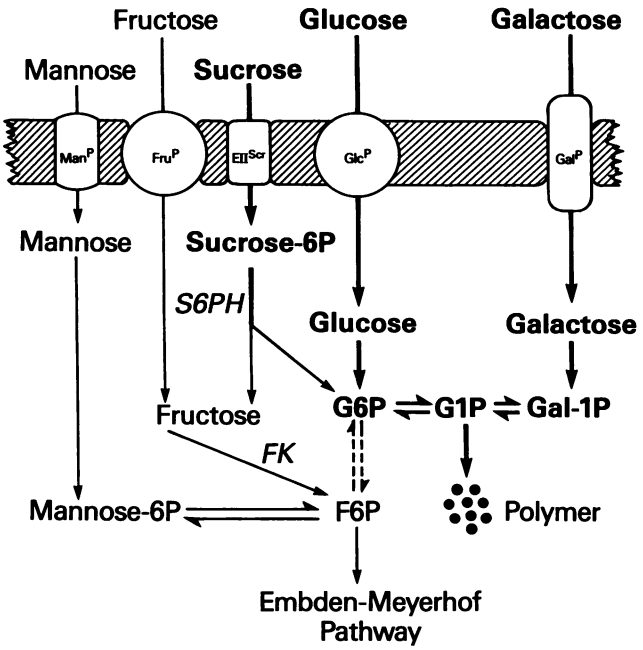

FIG. 5. Proposed pathways for transport, phosphorylation, and route(s) of polymer formation by *F. mortiferum* during growth on various sugars. Abbreviations: EII^{Scr}, enzyme II of sucrose-PEP-PTS; *S6PH*, sucrose 6-phosphate hydrolase; *FK*, ATP-dependent (manno)-fructokinase. Man^P, Fru^P, Glc^P, and Gal^P indicate putative sugar transporters for mannose, fructose, glucose, and galactose, respectively.

ACKNOWLEDGMENTS

We thank our colleagues J. London, P. E. Kolenbrander, and J. A. Donkersloot for advice and criticism during our investigation, and we express appreciation to J. Waters and O. Ambrose for preparing the electron micrographs. We thank Irma Gomez for technical assistance and Charlette Cureton for expert assistance in the preparation of the manuscript.

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