Anaerobic Transport in Escherichia coli Membrane Vesicles

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Anaerobic lactose and/or amino acid transport by membrane vesicles prepared from Escherichia coli ML 308-225 can be coupled to at least four electron transfer systems: α-glycerol-P dehydrogenase:nitrate reductase, formate dehydrogenase:nitrate reductase, α-glycerol-P dehydrogenase:fumarate reductase, and formate dehydrogenase:fumarate reductase. Vesicles contain one or more of these electron transfer systems depending on the growth conditions of the parent cells. α-Glycerol-P dehydrogenase and fumarate reductase are present only in vesicles prepared from cells grown in the presence of glycerol or fumarate, respectively. Formate dehydrogenase and nitrate reductase activities, on the other hand, are present in vesicles from cells grown on a variety of media. α-Glycerol-P and formate are able to drive aerobic transport in vesicles prepared from anaerobically grown cells, indicating coupling between aerobic and anaerobic electron transfer systems.

Active transport of β-galactosides and amino acids, as well as a variety of other metabolites, is coupled to electron transfer in cytoplasmic membrane vesicles isolated from Escherichia coli and a number of other organisms (1-4). In E. coli and Salmonella typhimurium membrane vesicles, the transport systems are coupled primarily to the oxidation of D-lactate or reduced phenazine methosulfate via a membrane-bound cytochrome chain with oxygen as the terminal acceptor (1-4). Recent experiments demonstrate that virtually all of the vesicles isolated from E. coli ML 308-225 catalyze active transport (5). Moreover, using antibodies directed against D-lactate dehydrogenase and calcium, magnesium-stimulated ATPase, it has been shown that these enzymes are localized on the inner surface of the vesicle membrane (6, 7). These and other findings (1-4, 8, 9) indicate that few, if any, of the vesicles are inverted, and, as such, support previous observations (1-4, 10, 11) which are consistent with the contention that the energy-coupling site for transport in E. coli vesicles is localized in a segment of the respiratory chain between D-lactate dehydrogenase and cytochrome b₅. The carriers, however, are not electron transfer intermediates (12), and are present in the membrane in large excess relative to D-lactate dehydrogenase (13). Recent evidence indicates that generation of a membrane potential is involved in the transport mechanism (4, 14, 15).

Previous studies (1-4, 16) indicate that ATP does not play a role in active transport in membrane vesicles. However, other evidence (17-24) is consistent with the hypothesis that glycolytically generated ATP is able to drive active transport in whole cells under anaerobic conditions. Recently, Konings and Kaback (25) demonstrated that anaerobic β-galactoside transport in whole cells and membrane vesicles from E. coli ML 308-225 is coupled to the oxidation of α-glycerol-P with fumarate as an anaerobic electron acceptor or to the oxidation of formate with nitrate as an anaerobic electron acceptor. In addition, Butlin (20) and Rosenberg et al. (26) have shown that mutants of E. coli which are deficient in calcium, magnesium-stimulated ATPase (uncA) are able to catalyze active transport of serine and phosphate under anaerobic conditions in the absence of fumarate as an electron acceptor.

The results presented in this paper demonstrate that in addition to β-galactosides, anaerobic amino acid transport is also driven by electron transfer in isolated membrane vesicles from E. coli ML 308-225. In addition, it is demonstrated that the β-galactoside and amino acid transport systems may be coupled to at least four distinct anaerobic electron transfer systems—α-glycerol-P dehydrogenase:fumarate reductase, α-glycerol-P dehydrogenase:nitrate reductase, formate dehydrogenase:fumarate reductase, and formate dehydrogenase:nitrate reductase.

METHODS

Cell Growth—Escherichia coli ML 308-225 (i⁻z⁻y⁻a⁻) was grown anaerobically at 37°C on Minimal Medium A supplemented with 0.1% yeast extract (Difco) as described previously (25). The following carbon sources, electron donors, and trace metals were added as indicated: (a)
0.5% glycerol plus 10 mM sodium fumarate; (b) 0.5% glycerol plus 50 mM potassium nitrate, 1 mM sodium selenate, and 1 mM sodium molybdate; (c) 0.5% glucose plus 50 mM sodium fumarate; and (d) 0.5% glucose plus 50 mM potassium nitrate, 1 mM sodium selenate, and 1 mM sodium molybdate. Appropriate controls (33) or nitrate-free, glucose-free, and lactose-free cells are referred to as “glycerol-selenate,” “glycerol-nitrate,” “fumarate-selenate,” and “glucose-nitrate” cells or vesicles throughout the paper.

Preparation of Spheroplasts and Membrane Vesicles—Cells were harvested at the end of exponential growth (A460 approximately 0.7 for glucose-grown cells and approximately 0.35 for glycerol-grown cells) after addition of chloramphenicol to a final concentration of 50 μg/ml. The cells were then resuspended in 30 mM Tris-HCl (pH 8.0) containing 20% sucrose and 50 μM/ml of chloramphenicol (10 g of cells, wet weight/80 ml of medium), and converted to spheroplasts by means of the lysozyme-ethylenediaminetetraacetic acid method (29). Membrane vesicles were prepared and isolated by the method described previously (25). Purified vesicles were resuspended in 50 mM potassium phosphate (pH 6.6) to a protein concentration of 5 to 10 mg/ml. Membranes of 0.5 to 1.0 ml were frozen rapidly and stored in liquid nitrogen.

Transport Assays—Transport under aerobic and anaerobic conditions was assayed as described previously (29, 30) with the exception that oxygen-free nitrogen rather than argon was used for the anaerobic assays. Lactose transport was assayed with [14C]lactose (30 mM Ci/mol) at a final concentration of 0.4 mM. Amino acid uptake was monitored with [14C]phenylalanine (50 mM Ci/ml and mixed in 20 mM sodium molybdate, 50 mM glucose, and 1 mM sodium [2,3-14C]fumarate (specific activity, 39 mCi/mmol). Samples were incubated at 25°C under nitrogen for 5 min. At this time, the reactions were terminated by addition of 10 μl of 2 N sodium perchlorate, and 10 μl of the inactivated suspension were then spotted on Silica Gel G thin layer plates (Merck, Darmstadt, West Germany). The plates were developed with water-saturated ether/ethanol/acetic acid (70:28:2, v/v). The lamina containing the amino acid mixture was scraped off, washed with 1 M NaOH, and counted for radioactivity. Reactions were terminated by addition of 10 μl of 0.1 N sodium perchlorate, and 10 μl of the inactivated suspension were then spotted on Silica Gel G thin layer plates (Merck, Darmstadt, West Germany). The plates were developed with water-saturated ether/ethanol/acetic acid (70:28:2, v/v). The lamina containing the amino acid mixture was scraped off, washed with 1 M NaOH, and counted for radioactivity.

Enzyme Assays—Spectrophotometric assays of α-glycerol-P dehydrogenase and DPNH reductase, and nitrate reductase was performed at 25°C using a Hitachi Perkin-Elmer double beam recording spectrophotometer (model 124, Tokyo Ltd., Japan).

Anaerobic α-glycerol-P dehydrogenase was assayed by phenazine methosulfate-mediated reduction of 3(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide in 370 μM as described by Lester and DeMoss (32). The extinction coefficient of reduced dichlorophenolindophenol was taken to be 7.4 mM-1 cm-1 at 570 nm (32). Activities are expressed as nanomoles per min per mg of membrane protein.

Fumarate reductase was performed at 25°C using a Hitachi Perkin Elmer double beam recording spectrophotometer (model 124, Tokyo Ltd., Japan). The extinction coefficient of reduced MTT was taken to be 17 mM-1 cm-1 at 570 nm (32). Activities are expressed as nanomoles of nitrite formed per min per mg of membrane protein.

Oxygen Consumption Measurement.—Oxygen consumption was measured polarographically with a Clark-type oxygen electrode (YSI model 53, Yellow Springs Instruments Co., Yellow Springs, Ohio) connected to a Servogor recorder (model RE 511) as described previously (36).

Protein—Protein was assayed by the method of Lowry et al. (37).

Materials—[14C]Lactose (20 Ci/mmol) was obtained from Amer sham/Searle (Arlington Heights, Ill.), and (2-C)aminom acid mixture and [2,3-14C]fumarate were obtained from the Radiocchemical Centre (Amersham). All other chemicals were reagent grade obtained from commercial sources. Stock solutions (0.5 ml) of electron donors and acceptors were neutralized to pH 7.0 with sodium or potassium hydroxide as indicated.

RESULTS

Anaerobic Transport by Anaerobically Grown Whole Cells

E. coli ML 308-225 grown anaerobically on media containing glycerol or glucose as carbon sources and fumarate or nitrate as anaerobic electron acceptors takes up amino acids effectively under anaerobic assay conditions (Fig. 1). The rate and extent of lactose uptake, on the other hand, appear to vary depending upon the carbon source and anaerobic electron acceptor used for growth, even though this mutant is constitutive for the β-galactoside transport system. Thus, anaerobic lactose transport is maximal in cells grown anaerobically on glycerol and fumarate, approximately 2-fold less active in cells grown anaerobically on glucose and fumarate, approximately 4-fold less active in cells grown anaerobically on glycerol and nitrate, and almost completely absent in cells grown anaerobically on glycerol and nitrate. Although not shown, it is noteworthy that cells grown aerobically on glycerol in the presence of nitrate transport lactose effectively under both aerobic and anaerobic conditions, suggesting that the low level of lactate transport observed here is not due solely to catabolite repression of lac carrier protein synthesis.

Active Transport by Membrane Vesicles Prepared from Anaerobically Grown Cells

Glycerol-Fumarate Vesicles—Membrane vesicles prepared from E. coli ML 308-225 grown anaerobically on glycerol and

*The abbreviation used is: MTT, 3(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide.
fumarate catalyze anaerobic lactose transport when supplied with three combinations of electron donors and acceptors—\( \alpha \)-glycerol-P and fumarate, \( \alpha \)-glycerol-P and nitrate, and formate and nitrate (Fig. 2, top). Very little or no stimulation is observed with formate and fumarate, or with \( \alpha \)-glycerol-P, formate, nitrate, or fumarate alone. However, as reported previously (25), in the absence of added electron donors or acceptors, relatively high endogenous uptake is observed.

When the same vesicles are assayed under aerobic conditions (Fig. 2, middle), the effect of the electron donors and acceptors is quite different. As shown, neither fumarate nor nitrate has a significant effect on lactose transport with \( \alpha \)-glycerol-P as the electron donor and oxygen as acceptor. With formate as electron donor, however, nitrate and especially fumarate enhance the rate and extent of lactose uptake over that observed with oxygen alone as the terminal acceptor.

As shown in the bottom of Fig. 2, glycerol-fumarate vesicles do not catalyze amino acid transport effectively under anaerobic conditions. Slight stimulation of uptake is observed with \( \alpha \)-glycerol-P or formate as electron donors and nitrate as electron acceptor. Moreover, under aerobic conditions, amino acid uptake by these vesicles is also relatively small (data not shown).

Glycerol-Nitrate Vesicles—Membrane vesicles prepared from \( E. \ coli \) ML 308 225 grown anaerobically with glycerol as carbon source and nitrate as electron acceptor catalyze amino acid transport under anaerobic conditions in the presence of formate and nitrate (Fig. 3, top). Formate and fumarate, \( \alpha \)-glycerol-P and nitrate, or \( \alpha \)-glycerol-P and fumarate produce much lower rates and extents of uptake. Addition of \( \alpha \)-glycerol-P or formate in the absence of an electron acceptor results in only a slight increase in amino acid transport under these conditions, and addition of fumarate or nitrate as electron acceptors in the presence of oxygen has no significant effect.

When amino acid uptake by these vesicles is assayed under aerobic conditions (Fig. 3, middle), significant stimulation is observed in the presence of \( \alpha \)-glycerol-P and formate. Moreover, addition of fumarate or nitrate as electron acceptors in the presence of oxygen has no significant effect.

Anaerobic lactose uptake in these vesicles is very low and no stimulation is observed with any combination of electron donors and acceptors tested (Fig. 3, bottom). This finding is consistent with experiments carried out with whole cells grown under these conditions (Fig. 1).

Glucose-Fumarate Vesicles—Although cells grown on glucose and fumarate transport lactose moderately well (Fig. 1), vesicles from these cells do not accumulate lactose effectively.
FIG. 3. Uptake of lactose and amino acids under anaerobic and aerobic conditions in membrane vesicles of anaerobically grown E. coli ML 308-225 on glycerol-nitrate medium. Transport assays and additions were performed as described in legend of Fig. 2.

Under anaerobic conditions, formate and nitrate and even nitrogen can formate as electron donor and acceptor, respectively (Fig. 3, top). The reason for this inconsistency is not apparent. Under aerobic conditions, however, the vesicles exhibit moderate activity formate as electron donor (Fig. 4, middle), and addition of fumarate or nitrate has no additional effect. No stimulation of aerobic lactose uptake in these vesicles is observed with α-glycerol-P in the presence or absence of fumarate or nitrate.

Amino acid uptake by these vesicles under anaerobic conditions is mildly stimulated by formate in the presence of formate, and even less so by formate in the presence of nitrate (Fig. 4, bottom). α-Glycerol-P with formate or nitrate as acceptors stimulates anaerobic amino acid uptake slightly or not at all, and no significant uptake is observed with electron donors or acceptors alone.

Glucose-Nitrate Vesicles—As shown in Fig. 5, vesicles prepared from cells grown anaerobically with glucose and nitrate catalyze anaerobic amino acid transport in the presence of formate and nitrate only (top panels). All other electron donors and acceptors, when present alone or in various combinations, have no significant effect on amino acid uptake.

Formate is also the only effective electron donor for aerobic amino acid uptake in these vesicles, and under these conditions, nitrate or fumarate produces no additional stimulation of amino acid uptake (middle panels).

Anaerobic lactose transport is also catalyzed by these vesicles in the presence of formate and nitrate, but the absolute amount of lactose transported is relatively low (bottom panels). As shown for amino acid transport, all other electron donors and acceptors alone or in various combinations have no significant effect on lactose uptake.

Enzyme Assays and Coupled Activities

The data presented in Table I demonstrate that formate dehydrogenase and nitrate reductase activities are present in all of the membrane vesicles used in these experiments, although the activities vary depending upon the growth conditions of the parent cells. Thus, formate dehydrogenase exhibits the highest activity in vesicles from cells grown on glucose in the presence of formate, and the lowest activity in vesicles prepared from cells grown on glycerol and formate.

Nitrate reductase activity, on the other hand, is highest in
glycerol-nitrate vesicles, and lowest in glucose-fumarate vesicles. Anaerobic \(\alpha\)-glycerol-P dehydrogenase activity is present only in vesicles prepared from glycerol-grown cells, and higher activity is observed when the cells are grown with fumarate as opposed to nitrate as an electron acceptor. Similarly, fumarate reductase activity is observed only when the cells are grown on fumarate, and activity is best when glycerol is used as the carbon source.

Membrane vesicles prepared from cells grown anaerobically on all of the media described exhibit coupling between formate dehydrogenase and nitrate reductase (Table II). Coupled activity between these enzymes is highest in vesicles prepared from glycerol-grown cells, and intermediate in glycerol-fumarate and glycerol-nitrate vesicles, and lowest in glucose-fumarate vesicles. Coupling between formate dehydrogenase and fumarate reductase is not present in any of the vesicle preparations, a finding which is consistent with observations demonstrating that none of the vesicles catalyzes anaerobic lactose or amino acid transport in the presence of formate and fumarate. Significant coupling between \(\alpha\)-glycerol-P dehydrogenase and nitrate reductase is observed only in vesicles from cells grown on glycerol. It is surprising, moreover, that this activity is better when the cells are grown with fumarate as an electron acceptor as opposed to nitrate. Finally, coupling between \(\alpha\)-glycerol-P dehydrogenase and fumarate reductase is observed only in vesicles from cells grown on glycerol with fumarate as an anaerobic electron acceptor, a finding which is consistent with the observations that only these vesicles exhibit both \(\alpha\)-glycerol-P dehydrogenase and fumarate reductase activity (Table I).

The data presented in Table III demonstrate that formate is oxidized by all of the vesicle preparations (although formate oxidation is quite low in glucose-fumarate vesicles), a finding which is consistent with observations demonstrating that all of the vesicles exhibit formate dehydrogenase activity (Table I) and that formate drives lactose or amino acid transport under aerobic conditions. \(\alpha\)-Glycerol-P is oxidized only by glycerol-fumarate and glycerol-nitrate vesicles, both of which contain \(\alpha\)-glycerol-P dehydrogenase (Table I) and exhibit \(\alpha\)-glycerol-P-driven lactose or amino acid transport under aerobic and anaerobic conditions. These results confirm the observation that oxygen can serve as a terminal electron acceptor in vesicles from certain anaerobically grown cells.

Effect of Ascorbate-Phenazine Methosulfate, \(d\)-Lactate, Succinate, and NADH on Transport under Aerobic Conditions

Active transport of a number of solutes, including lactose and amino acids, by membrane vesicles prepared from \(E.\ coli\) ML 308-225 grown aerobically is coupled primarily to the oxidation of \(d\)-lactate or reduced phenazine methosulfate (1–4). Succinate and NADH also drive transport to some extent, although they are generally much less effective than

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**Table I**

| Vesicles          | FDH a | \(\alpha\)-GPDH anaerobic c | NR d | FR e |
|-------------------|-------|-----------------------------|------|------|
|                   | nmol/min/mg membrane protein |       |      |      |
| Glycerol-fumarate | 70    | 50                          | 2630 | 1190 |
| Glycerol-nitrate  | 220   | 20                          | 2830 | 10   |
| Glucose-fumarate  | 550   | 0                           | 200  | 550  |
| Glucose-nitrate   | 150   | 0                           | 1830 | 10   |

a Enzyme activities were assayed as described under "Methods."  
b Formate dehydrogenase.  
c Anaerobic \(\alpha\)-glycerol-P dehydrogenase.  
d Nitrate reductase.  
e Fumarate reductase.

**Table II**

| Coupled enzyme activities a | FDH:NR | FDH:FR | \(\alpha\)-GPDH:NR d | \(\alpha\)-GPDH:FR e |
|-----------------------------|--------|--------|---------------------|---------------------|
| Vesicles                    | nmol/min/mg membrane protein |        |                     |                     |
| Glycerol-fumarate           | 109    | 0      | 48                  | 1161                |
| Glycerol-nitrate            | 65     | 0      | 16                  | 0                   |
| Glucose-fumarate            | 27     | 0      | 0                   | 0                   |
| Glucose-nitrate             | 150    | 0      | 2                   | 0                   |

a Coupled activities were measured as described under "Methods."  
b Formate dehydrogenase:nitrate reductase.  
c Formate dehydrogenase:fumarate reductase.  
d \(\alpha\)-Glycerol-P dehydrogenase:nitrate reductase.  
e \(\alpha\)-Glycerol-P dehydrogenase:fumarate reductase.

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Fig. 5. Uptake of lactose and amino acids under anaerobic and aerobic conditions in membrane vesicles of anaerobically grown \(E.\ coli\) ML 308-225 on glucose-nitrate medium. Transport assays and additions were performed as described in legend of Fig. 2.
p-lactate or reduced phenazine methosulfate. None of these electron donors stimulates anaerobic lactose or amino acid transport in vesicles prepared from cells grown anaerobically under any of the conditions described in this paper, even in the presence of nitrate or fumarate (data not shown). However, as shown in Fig. 6, when the vesicle preparations used in these experiments are assayed under aerobic conditions, many of these electron donors drive lactose or amino acid uptake effectively. Thus, lactose transport and amino acid transport are markedly stimulated by ascorbate-phenazine methosulfate in glycerol-fumarate vesicles, and glycerol-nitrate, glucose-fumarate, and glucose-nitrate vesicles, respectively. Interestingly, p-lactate does not drive lactose or amino acid transport aerobically except in glycerol-nitrate vesicles. Moreover, NADH is able to drive lactose or amino acid uptake reasonably well in all of these vesicles. Finally, it is noteworthy that succinate drives lactose transport at the same rate as ascorbate-phenazine methosulfate in glycerol fumarate vesicles, but is much less effective than ascorbate-phenazine methosulfate for amino acid transport in the other vesicle preparations.

**DISCUSSION**

The data presented in this paper confirm and extend previous studies (25) on active transport under anaerobic conditions in isolated membrane vesicles from *E. coli*. As shown, lactose and/or amino acid transport can be coupled to at least four electron transfer systems which do not require oxygen as the terminal electron acceptor. These anaerobic electron transfer systems are α-glycerol-P dehydrogenase:fumarate reductase, α-glycerol-P dehydrogenase:nitrate reductase, formate dehydrogenase:nitrate reductase, and formate dehydrogenase:fumarate reductase. The α-glycerol-P dehydrogenase:fumarate reductase system has been studied in detail by Miki and Lin (38), and the involvement of a b-type cytochrome(s) was suggested by Konings and Kaback (25). The formate dehydrogenase:nitrate reductase system has been studied extensively by Ruiz-Herrera et al. (39) and others (27, 40-43).

Anaerobic growth on glycerol induces an anaerobic α-glycerol-P dehydrogenase (32, 44), and addition of fumarate as an electron acceptor induces fumarate reductase (33, 45). The synthesis of fumarate reductase is inhibited by the presence of nitrate in the growth medium (46). Alternatively, anaerobic growth on glucose with nitrate as an electron acceptor induces formate dehydrogenase and nitrate reductase (27). In addition, the latter two enzymes require selenium and molybdate for maximal activity (28, 47). Nitrate reductase has been shown to accept electrons from a cytochrome of the b-type (39, 42), and addition of nitrate to the growth medium inhibits the biosynthesis of a soluble formate dehydrogenase which is involved in the hydrogenlyase pathway (40).

α-Glycerol-P dehydrogenase:fumarate reductase is present only in vesicles prepared from cells grown anaerobically on media containing glycerol and fumarate. Such vesicles catalyze anaerobic lactose transport in the presence of α-glycerol-P and fumarate. The formate dehydrogenase:nitrate reductase system is present in vesicles prepared from cells grown anaerobically on media containing glucose and nitrate as reported previously (25), but also in vesicles prepared from cells grown anaerobically on glycerol and nitrate, glucose and fumarate, and glycerol and fumarate. Not only are the appropriate enzymes present, but addition of formate and nitrate drives anaerobic lactose and/or amino acid transport in all of the vesicle preparations except those prepared from cells grown on glucose and fumarate. The reason for the lack of activity in the latter preparation may be due to the relatively low level of nitrate reductase present in these vesicles. In any case, it is apparent that significant levels of formate dehydrogenase and nitrate reductase are produced in cells grown anaerobically under each of the conditions described. It should be emphasized, however, that yeast extract was added to all of the media, and it is possible that this supplement contains the factors necessary for the synthesis of these enzymes.

The α-glycerol-P dehydrogenase:nitrate reductase system is

**Table III**

Oxidation rates of various substrates in membrane vesicles from anaerobically grown *Escherichia coli* ML 308-225

| Electron donors acceptors (10 mM) | Oxygen Consumption* | Glycerol-fumarate vesicles | Glycerol-nitrate vesicles | Glucose-fumarate vesicles | Glucose-nitrate vesicles |
|----------------------------------|----------------------|---------------------------|--------------------------|--------------------------|-------------------------|
| Formate                          | 135                  | 65                        | 7                        | 33                       |
| Formate + fumarate               | 120                  | 62                        | 5                        | 20                       |
| Formate + nitrate                | 96                   | 53                        | 5                        | 17                       |
| α-Glycerol-P                     | 20                   | 27                        | 0                        | 1                        |
| α-Glycerol-P + fumarate          | 12                   | 18                        | 0                        | 1                        |
| α-Glycerol-P + nitrate           | 15                   | 11                        | 0                        | 1                        |

*Oxygen consumption was measured as described under "Methods."
present only in glycerol-fumarate and glycerol-nitrate vesicles, and this anaerobic electron transfer system is able to drive both lactose and amino acid transport under anaerobic conditions. Formate dehydrogenase and fumarate reductase activities are observed in glucose-fumarate and glycerol-fumarate vesicles, but coupling between these enzymes could not be demonstrated.

It is apparent from these studies that formate dehydrogenase and/or α-glycerol-P dehydrogenase are also able to drive active transport in vesicles prepared from anaerobically grown cells with oxygen as the terminal electron acceptor. This finding suggests that there must be some degree of coupling between anaerobic and aerobic electron transfer chains in the vesicle membrane. In contrast to vesicles prepared from aerobically grown cells where n-lactate and reduced phenazine methosulphonium and rubidium (in the presence of valinomycin) (4, 49) are able to drive transport under anaerobic conditions. Similarly, NADH and succinate are not able to drive transport under anaerobic conditions. However, when vesicles prepared from anaerobically grown cells are assayed under aerobic conditions, o-lactate is no longer the best physiological electron donor for active transport, and succinate or NADH functions more effectively in this capacity. Since recent experiments provide convincing evidence that the generation of a membrane potential is intimately involved in active transport (4, 14, 48), it seems quite possible that variations in the efficiency of different electron donors to drive active transport under anaerobic or aerobic conditions may reflect variations in their ability to generate the appropriate membrane potential. Measurement of membrane potentials with triphenylmethylphosphonium and rubidium (4, 49) under the conditions described here is currently in progress.

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