Energy Coupling of the \( \beta \)-Methylgalactoside Transport System of Escherichia coli*

(Received for publication, November 3, 1972)

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SUMMARY

Energy coupling for the concentrative uptake of galactose via the \( \beta \)-methylgalactoside transport system occurs via an intermediate produced during oxidative phosphorylation but before ATP formation. The membrane-bound ATPase, necessary for ATP formation during oxidative phosphorylation, is not obligatory for energy coupling of transport but can, under anaerobic conditions, accomplish energy coupling by ATP hydrolysis. These conclusions are supported by the following findings: (a) galactose uptake in intact cells can be stimulated by substrates of the respiratory chain and inhibited by inhibitors of the respiratory chain and by uncouplers of oxidative phosphorylation; (b) cells made anaerobic for the transport assay are fully transport active; (c) a mutant defective in the membrane-bound ATPase has lost transport activity unless supplied with exogenous D-lactate, and the additional presence of arsenate completely blocks the stimulation by D-lactate; (d) a mutant defective in the respiratory chain due to a defect in ubiquinone biosynthesis still exhibits transport activity, which cannot be stimulated by D-lactate.

The accumulation of substances within a bacterial cell against the concentration gradient requires an expenditure of energy. Despite many recent studies the molecular mechanism by which energy coupling to bacterial active transport systems is accomplished remains an intriguing problem.

Several different proposals have been made for the energy source of the lactose transport system. Scarborough et al. (1) reported that ATP, under conditions where the cells were made permeable to this compound, can increase the rate of downhill transport of ONPGal, indicating that energy could be derived aerobically by oxidative phosphorylation or anaerobically by glycolysis. Recently Schairer and Haddock (2) studied the same transport system in mutants defective in the membrane-bound Mg\(^{2+}\), Ca\(^{2+}\)-stimulated ATPase and could show that not ATP itself but rather an intermediate of oxidative phosphorylation is necessary for energy coupling of \( \beta \)-galactoside transport. The intermediate can be derived either by oxidation of a suitable substrate via the respiratory chain or by ATP hydrolysis via the membrane-bound ATPase. Studying amino acid transport in mutants defective in electron transport and the Mg\(^{2+}\), Ca\(^{2+}\)-stimulated ATPase, Simoni and Shallenberger (3) came to the same conclusion.2 Klein and Boyer's (4) recent inhibitor studies of sugar and amino acid transport in *Escherichia coli* also indicated that either phosphate bond or oxidative energy could be used to drive active transport. Pavlasova and Harold (5) found that uncouplers of oxidative phosphorylation inhibited TMG accumulation under anaerobic conditions while the steady state level of ATP remained constant. This observation led them to suggest that the energy source for the lactose system might be linked to a proton or ion gradient across the cell membrane in accordance with the chemiosmotic hypothesis of energy coupling originally proposed by Mitchell (6). The chemiosmotic coupling mechanism has received additional support from West (7, 8), who reported that poisoned cells take up protons concomitant with the downhill transport of lactose. Recent studies from Kaback's laboratory (9–16) have provided an entirely new approach to the problem of energy coupling in bacteria. Using membrane vesicles devoid of cytoplasm and incapable of oxidative phosphorylation, Kaback and his colleagues (10, 11) could demonstrate that concentrative uptake of lactose as well as other sugars and amino acids is coupled primarily to a membrane-bound,2

* This work was supported by grants from the National Institutes of Health (GM-18498) and the National Science Foundation (GB-38785X).
† In partial fulfillment of the requirements for the Degree of Bachelor of Arts in Biochemistry.
1 The abbreviations used are: ONPGal, o-nitrophenyl-\( \beta \)-galactopyranoside; TMG, methyl-1-thio-\( \beta \)-D-galactopyranoside. All other sugars mentioned in the text have \( \alpha \)-configuration. p-HMB, \( \beta \)-hydroxymercaptobenzoate; CCCP, carbonyl cyanide m-chlorophenylhydrazone.
2 Studies of active transport in ATPase-deficient mutants have led to somewhat confusing results. The uptake of TMG, proline, serine, and lysine by whole cells and membrane vesicles of AN120 has been reported to be normal in a recent study from Kaback's laboratory (16). Schairer and Haddock (2) also showed normal uptake of TMG in a different Mg\(^{2+}\), Ca\(^{2+}\)-stimulated ATPase mutant of *E. coli*. Simoni and Shallenberger (3) have reported a decreased ability to transport proline and alanine in another *E. coli* strain deficient in this enzyme. Kaback (15) has suggested that the ATPase mutant studied by Simoni and Shallenberger may have more than one defect. Discrepancies in the basal rates of transport, i.e., in the absence of inhibitors or energy sources, for substrates of different transport systems may be a reflection of the relative degree of dependency of these systems upon each of the two pathways by which energy can be derived for transport.
flavin-linked n-lactate dehydrogenase, but not to NADH dehydrogenase. Therefore, active transport of lactose in membrane vesicles seems to depend on electron transport but not upon the participation of a high energy intermediate of oxidative phosphorylation. From these data a model was proposed for the active transport of lactose and other substrates; the carrier molecules were thought to be obligatory intermediates of the respiratory chain located between the n-lactate dehydrogenase and cytochrome b1 (11, 12).

However, the isolation of electron transfer coupling mutants, which oxidize n-lactate and other electron donors at a normal rate but cannot catalyze respiration-linked transport, necessitated the modification of this model in order to account for electron transfer in the absence of active transport (15). It was therefore suggested that the transport carrier molecules may be components of shunts off the main part rather than obligatory intermediates of the respiratory chain (15).

While studies with membrane vesicles of E. coli have contributed to our understanding of energy coupling of the lactose transport system, as well as other sugar and amino acid transport systems, they seem not to be as useful in the study of transport systems involving periplasmic binding proteins. Vesicles of E. coli ML3 and ML35 have lost their galactose-binding protein together with a high affinity transport system for galactose, the β-methylgalactoside transport system (14). Any attempt to restore transport activity of the β-methylgalactoside transport system by addition of purified galactose-binding protein to membrane vesicles has failed so far. Neither has it been possible to demonstrate any binding of radioactively labeled galactose-binding protein to membrane vesicles. In the present paper we attempted to study the energy coupling of the β-methylgalactoside transport system mediated by the periplasmic galactose-binding protein in whole cells.

**Materials and Methods**

**Bacterial Strains**

Unless otherwise specified all tests were performed with strain W3912gy(−) (ATCC 25933) (F−, galK, lacY). This strain is endogenously induced for the β-methylgalactoside transport system and the synthesis of the galactose-binding protein and has been described (18). Four strains were obtained from Dr. Cox: AN180 (F−, argE3, thi-1, strR) (19), AN120 (F−, argE3, thi-1, strR, uncA6) (19), AR2154 (Hfr, metF, thr-1, leu-d) (20), AN359 (Hfr, thr-1, leu-d, ubiB+) (20). Before using these strains, single cell colonies were isolated and tested for their amino acid markers by growth on minimal plates containing 0.2% glucose in the presence and absence of the respective amino acid. The uncA301 and the ubiB markers were tested by the ability of the strains to grow aerobically on n-lactate or succinate as sole carbon source. It was noted that strain AN59 exhibits a high reversion rate to ubiB−. Therefore, after measuring β-methylgalactoside transport activity, the ubiB marker had to be rechecked routinely to avoid misinterpretation caused by the outgrowth of ubiB− reversions.

**Growth of Bacterial Strains and Preparation of Bacterial Suspension Used for Transport Assay**

All strains were grown and prepared for the transport assay as previously described (21).

1. W. Boos, A. S. Gordon, H. R. Kabaek, unpublished observation.

2. Genetic symbols are according to Taylor (17).

**Transport Assay**

**Initial Rate of Entry of Galactose**—The assay for the initial rate of galactose entry was performed as described elsewhere (21) with the modifications indicated below. When energy poisons were used during the assay, they were added in maximally 100 μl of concentrated solutions either together with the radioactive galactose or 10 min prior to the addition of labeled galactose. Substrates to stimulate transport were added routinely 5 min prior to the addition of labeled galactose. Some components with limited solubility in water were dissolved in maximally 100 μl of dimethyl sulfoxide, which by itself did not change transport activity. When uptake under anaerobic conditions was measured, the cell suspension prepared for the transport test was incubated at 37°C for up to 3 hours in the presence of chloramphenicol (100 μg per ml) while oxygen-free helium was bubbled through the suspension continuously. After temperature equilibrium to the assay temperature 5 ml were quickly removed and transferred into a vessel filled with CO2 and containing 50 μl of [1-14C]galactose, resulting in a final concentration of 0.5 μM. Aliquots (0.5 ml) were removed as quickly as possible and filtered as usual.

**Initial Rate of Exit of Galactose**—The assay for the initial rate of exit of galactose has been described (21). To measure the effect of n-lactate on exit of preaccumulated [1-14C]galactose, n-lactate was added to the 10-ml test suspension of preloaded cells in 20 μl of concentrated solution 5 min prior to the addition of excess unlabeled sugar or energy poisons. When energy poisons were utilized in the exit assay, they were added to the test suspension in 50 μl of solution at time 0.

**Combined Treatment of Temperature and Osmotic Shock to Increase Permeability of ATP**

The following procedure was done according to a suggestion of Dr. M. Cashel, National Institutes of Health. After growth in nutrient broth the bacterial cells were washed once with Medium A at 25-30°C and centrifuged at the same temperature. They were resuspended in 0.1 M Tris-HCl, pH 7.3, at room temperature and centrifuged at 25-30°C. The supernatant was carefully removed and the cell pellet was resuspended in ice-cold 1 mM Tris-HCl, pH 7.3, containing 1 mM MgCl2. The cells were centrifuged at 0-4°C and resuspended in ice-cold Medium A to an optical density of 0.5 at 650 nm. For the transport test this suspension was then treated as described above.

**Results**

**Effect of Different Energy Poisons on Initial Rate of Galactose Uptake**—Table 1 shows the ability of different substances to interfere with the initial rate of entry of galactose at 23°C. Uncouplers of oxidative phosphorylation as well as inhibitors of glycolysis show varying degrees of inhibition. Surprisingly, oligomycin, an inhibitor of the Mg2+-, Ca2+-stimulated ATPase in mitochondria, causes a reproducible stimulation of transport. Most of the inhibitors are temperature-dependent in their inhibitory effect, being two to three times more effective at 23°C than at 15°C. The effect of 30 mM potassium cyanide is rather complex. Almost no inhibition of uptake is observed when it is added together with the [1-14C]galactose. The inhibitory effect increases with time and no transport activity can be observed after 10 min of incubation. This time dependence of potassium cyanide inhibition is similar to that previously described for p-HMB inhibition of galactose uptake (21). Yet in contrast to the effect of p-HMB,
glucose-stimulated exit of galactose from preloaded cells is not inhibited even after 3 min incubation with 30 mM potassium cyanide.

Effect of n-Lactate on Rate of Entry, Exit, and Steady State of Accumulation of Galactose—Fig. 1 shows that n-lactate stimulates the initial rate of uptake of galactose about 2-fold. The rate of exit as measured by addition of glucose, sodium azide, or CCCP to cells preloaded to an internal [1-14C]galactose concentration of 1 mM shows no significant difference whether or not n-lactate was present prior to the initiation of exit (Fig. 2). Therefore, n-lactate must act primarily on the entry process. This finding supports results obtained with energy poisons (21) and indicates energy coupling to the entry process. As a result of the increased rate of uptake in the presence of n-lactate, the ratio of internal to external galactose is increased in cells which have reached their steady state of accumulation. The addition of n-lactate results therefore in a 25 to 30% decrease of the external galactose concentration (Fig. 2D). The stimulation of initial rate of entry by n-lactate also shows a temperature dependence similar to that observed with energy poisons. Its maximum appears to be at 25°C.

Table I: Effect of energy poisons on initial rate of uptake of galactose at 23°C

| Additions            | Initial rate of uptake | Initial transport activity |
|----------------------|------------------------|----------------------------|
| None                 | 0.215                  | 135%                       |
| Oligomycin 0.1 mM    | 0.289                  | 108%                       |
| Antimycin A 40 mM    | 0.232                  | 105%                       |
| 2-Heptyl-4-hydroxyquinoline-N-oxide 40 mM | 0.221 | 105% |
| Ovomucoid 30 mM      | 0.189                  | 85%                        |
| Sodium amytal 2 mM   | 0.166                  | 78%                        |
| Sodium fluoride 30 mM | 0.141                  | 66%                        |
| Sodium arsenate 30 mM | 0.084                  | 39%                        |
| Sodium azide 30 mM   | 0.013                  | 6%                         |
| CCCP 40 μM           | 0.007                  | 3%                         |
| Dinitrophenol 2 mM   | 0.004                  | 2%                         |
| Potassium cyanide 30 mM | 0.003                | 1%                         |

Fig. 1. Effect of n-lactate on the uptake of galactose. Cells prepared for the transport assay were incubated at 25°C for 5 min. [1-14C]Galactose was added at time 0 to a final concentration of 0.5 μM. Aliquots (0.5 ml) were removed as fast as possible and filtered through Millipore filters (0.65 μm pore size) without washing and the filters were counted in a liquid scintillation counter. The total number of counts per min per 0.5-ml aliquot was 20,000. The results are given in nanomoles of galactose accumulated per 2.5 X 10⁶ cells or 50 μg dry weight of cells. Δ—Δ, control; ▲—▲, transport assay after addition of 20 mM n-lactate added 5 min prior to the addition of [1-14C]galactose.

Effect of Different Metabolites on Initial Rate of Galactose Uptake—Table II shows the ability of a variety of metabolites to stimulate the initial rate of uptake of galactose after incubation with cells for 5 min at 23°C. From the table it is clear that all metabolites which can serve as substrates of the respiratory chain, as well as those which can be easily converted into substrates, stimulated transport up to 3-fold. In contrast, ATP and other triphosphonucleosides are not able to stimulate; ATP and carbamylphosphate show a slight but significant and reproducible inhibition of uptake.

Treatments to Increase Permeability for ATP—To see whether or not the inability of cells to respond to ATP was caused by a transport barrier, cells were treated by different procedures...
state of accumulation of galactose. For measuring exit (A, B, C), cells prepared for the transport assay were incubated at room temperature with 0.5 μM [1-14C]galactose for 5 min. Exit of galactose was then initiated by 0.3 mM glucose (○ ○), 20 mM sodium azide (Δ−Δ), or 40 μM CCCP (□ −□), all final concentrations; filled symbols represent assays in the presence of 20 mM n-lactate added 5 min prior to the initiation of exit. To measure the steady state of accumulation (D), the cells were preloaded with 0.5 μM [1-14C]galactose at 25° for 5 min. n-Lactate was added and at times indicated 0.5-ml aliquots were filtered through Millipore filters (0.65 μm pore size). Aliquots (0.2 ml) of each filtrate were counted in a scintillation counter. The results are added and at times indicated 0.5-ml aliquots were filtered through Millipore filters (0.65 μm pore size). Aliquots (0.2 ml) of each filtrate were counted in a scintillation counter. The results are given as galactose concentration remaining in the external medium.

**FIG. 2. Effect of n-lactate on exit of galactose and on the steady state of accumulation of galactose.** For measuring exit (A, B, C), cells prepared for the transport assay were incubated at room temperature with 0.5 μM [1-14C]galactose for 5 min. Exit of galactose was then initiated by 0.3 mM glucose (○ ○), 20 mM sodium azide (Δ−Δ), or 40 μM CCCP (□ −□), all final concentrations; filled symbols represent assays in the presence of 20 mM n-lactate added 5 min prior to the initiation of exit. To measure the steady state of accumulation (D), the cells were preloaded with 0.5 μM [1-14C]galactose at 25° for 5 min. n-Lactate was added and at times indicated 0.5-ml aliquots were filtered through Millipore filters (0.65 μm pore size). Aliquots (0.2 ml) of each filtrate were counted in a scintillation counter. The results are given as galactose concentration remaining in the external medium.

**FIG. 3. Effect of a combined treatment of temperature and osmotic shock on the uptake of galactose.** Cells were treated as described under “Materials and Methods.” Transport assay was performed at 23° but otherwise as described in the legend to Fig. 1. C—C, no additions; ○—○, addition of 20 mM n-lactate 2 min prior to the addition of [1-14C]galactose; ●—●, addition of 20 mM ATP 2 min prior to the addition of [1-14C]galactose; ○—○, control, nonshocked cells.

**TABLE II**

**Effect of metabolites on initial rate of uptake of galactose at 23°**

Cells were incubated for 5 min at 23° with the metabolite prior to the addition of 0.5 μM [1-14C]galactose, final concentration.

| Additions in 20 mM solution unless otherwise indicated | Initial rate of uptake (μmol/min/cells) | Multifold stimulation |
|--------------------------------------------------------|----------------------------------------|---------------------|
| None                                                   | 0.215                                  | 2.5                 |
| Phosphoenolpyruvate                                     | 0.613                                  | 2.8                 |
| Pyruvate                                                | 0.600                                  | 2.7                 |
| Acetyl-CoA, 1 mM                                       | 0.581                                  | 2.7                 |
| dL-Hydroxyacetone                                      | 0.583                                  | 2.8                 |
| n-Lactate                                              | 0.582                                  | 2.7                 |
| d-Lactate                                              | 0.516                                  | 2.4                 |
| α-Ketoglutarate                                        | 0.515                                  | 2.3                 |
| NADH, 10 mM                                            | 0.514                                  | 2.3                 |
| Isoicatrate                                             | 0.510                                  | 2.3                 |
| Succinate                                               | 0.509                                  | 2.3                 |
| Dihydroxyacetone                                       | 0.476                                  | 2.2                 |
| TPN                                                     | 0.421                                  | 1.9                 |
| TPNH                                                    | 0.396                                  | 1.8                 |
| Acetylphosphate                                        | 0.331                                  | 1.5                 |
| NAD, 10 mM                                             | 0.326                                  | 1.5                 |
| Fructose 1,6-diphosphate                                | 0.285                                  | 1.3                 |
| Fructose 6-phosphate                                    | 0.285                                  | 1.3                 |
| 2,3-Diphosphoglycerate                                  | 0.245                                  | 1.1                 |
| 3-Phosphoglycerate                                      | 0.245                                  | 1.1                 |
| UTP                                                     | 0.231                                  | 1.0                 |
| CTP                                                     | 0.203                                  | 0.9                 |
| Cyclic 3',5'-AMP                                        | 0.199                                  | 0.9                 |
| Carbamyl phosphate                                     | 0.156                                  | 0.7                 |
| ATP                                                     | 0.144                                  | 0.7                 |
Galactose Uptake and Galactose-binding Protein Synthesis in Strains Defective in Membrane-bound ATPase and in Ubiquinone Biosynthesis—The finding that inhibitors of both oxidative phosphorylation and glycolysis are able to inhibit galactose transport suggested that energy derived from oxidative phosphorylation is a possible but not obligatory energy pathway. This idea was supported by the finding that cells kept anaerobic under helium at 37° for times up to 3 hours in the absence of any exogenous energy source and in the presence of chloramphenicol showed the same transport activity as a parallel cell suspension which was kept aerobic. It was therefore of interest to see whether or not enzymatic defects in the respiratory chain or oxidative phosphorylation would show any interference with transport activity of galactose. Two mutants were available, one defective in ubiquinone biosynthesis (20) and one defective in the membrane-bound Mg\(^{2+}\)-, Ca\(^{2+}\)-stimulated ATPase (19). Neither strain can grow on D-lactate or succinate, and both show reduced growth rates in nutrient broth. Fig. 4 shows the transport activity of the \(ub\) strain AN59 in comparison to its \(ub\) parent AB2154. As can be seen, the defect in ubiquinone biosynthesis reduces but does not abolish transport activity, and the addition of D-lactate to the mutant stimulates only to an insignificant extent. Arsenate inhibits the galactose uptake in both the mutant and its parent. The behavior of the ATPase defective mutant AN120 in comparison with its parent AN180 is shown in Fig. 5. The mutant exhibits only residual transport activity. However, the addition of D-lactate almost completely restores the initial rate of uptake to the value observed in the parent strain, while the additional presence of arsenate completely counteracts the stimulating effect of D-lactate. This clearly shows that in the absence of a functional ATPase, the energy necessary for the uptake of galactose against the concentration gradient can be derived from an intermediate generated during oxidative phosphorylation, but before ATP formation. It should be mentioned that both parents of the respective mutants show, in comparison with our wild type W3092cy\(^{-}\), a rather low transport activity, which cannot be further induced by the presence of D-fucose during growth. However, neither mutation affects the amount of galactose-binding protein synthesized, in comparison with its respective parent, as judged by the ability of toluenized cells to cross-react with antigaalactose-binding protein antibodies (Fig. 6). Again, this amount is smaller than that observed in our wild type W3092cy\(^{-}\), and might account for the relatively low transport activity of these strains.
DISCUSSION

The results presented here indicated that energy for the concentrative uptake of galactose mediated by the β-methylgalactoside transport system is derived from a high energy intermediate which can be generated either during oxidative phosphorylation before the formation of ATP or directly from ATP hydrolysis, via the Mg$^{2+}$-, Ca$^{2+}$-stimulated, membrane-bound ATPase.

It was found that substrates of the respiratory chain or metabolites which can be converted into into respiratory substrates stimulate transport up to 3-fold. Although this effect does not reveal the site at which energy coupling occurs, it does indicate that glycolysis itself is not required.

A variety of energy poisons were shown to be potent inhibitors of the initial rate of galactose uptake. The most effective were the respiratory inhibitor cyanide and the uncouplers of oxidative phosphorylation: dinitrophenol, CCCP, azide, and arsenate. The inhibition by uncoupling agents indicates that respiration is not sufficient for the accumulation of galactose. It was also observed that aerobically grown cells could be incubated anaerobically for up to 3 hours (in the absence of protein synthesis) without reduction of their ability to actively transport galactose. Taken together these inhibitor studies suggest that energy can be derived from an intermediate of oxidative phosphorylation under aerobic conditions or from glycolysis (via ATP hydrolysis) under anaerobic conditions. However, it should be noted that the strong inhibition of galactose uptake by cyanide does not appear consistent with the failure of anoxia to reduce transport activity. In addition, we were not able to directly demonstrate any stimulation of transport by exogenous ATP even under conditions which are known to allow the penetration of nucleoside triphosphates (23, 26). ATP showed a slight but reproducible inhibition of transport as observed by Knappe et al. (27) for the uptake of arginine by E. coli membranes. The failure of ATP to stimulate transport might possibly be explained by a strong feedback regulation of transport activity by the energy charge of the cell, i.e. the ratio: $((\text{ATP}) + 1/2(\text{ADP}))/((\text{ATP}) + (\text{ADP}) + (\text{AMP}))$ (28). One would therefore expect transport activity to be high at a low energy charge in order to accumulate more metabolizable substrates, and low at a high energy charge, i.e. an inhibitory effect by a high ATP concentration.

The strongest evidence for the conclusions concerning energy coupling are derived from measurements of galactose uptake in mutants defective in oxidative phosphorylation. One mutant, AN59, which is defective in ubiquinone biosynthesis, is unable to grow on n-lactate or succinate because of its respiratory chain deficiency. This strain also exhibits a reduced galactose transport activity which cannot be significantly stimulated by n-lactate. Since the mutant cannot derive energy from respiration or oxidative phosphorylation, it is dependent upon the production of ATP via glycolysis for all energy-requiring processes. Thus, its reduced transport activity reflects the loss of one of the two methods of generating energy which can be coupled to galactose accumulation. Furthermore, it was shown that the residual transport activity of this mutant was inhibited by the phosphate analogue arsenate. This inhibition is expected based on the demonstrated ability of arsenate to reduce intracellular ATP and phosphoenolpyruvate levels in intact cells of E. coli (4).

The other mutant studied, AN120, can oxidize metabolites via the electron transport chain but is deficient in the Mg$^{2+}$-, Ca$^{2+}$-stimulated, membrane-bound ATPase. Thus, the failure of this strain to grow on n-lactate or succinate results from its inability to couple respiratory chain oxidation to ATP formation. As a result, no energy for galactose uptake can be generated from ATP. This mutant was shown to be almost completely devoid of transport activity in the absence of added energy source. However, n-lactate stimulates galactose uptake to the level observed in the parent strain, indicating that the effect of this metabolite is not mediated by the generation of ATP. It was also shown that arsenate completely blocks the stimulatory effect of n-lactate, demonstrating that respiration alone is not enough for n-lactate to influence transport. Arsenate acts as a "secondary" uncoupler of oxidative phosphorylation; although its precise mechanism of action is unknown, it is thought to replace phosphate by forming an unstable arsonyl intermediate which is then spontaneously hydrolyzed (29). Arsenate could therefore discharge an energy-rich intermediate of oxidative phosphorylation even in the absence of the enzyme responsible for the final step(s) in the generation of ATP. Such a mode of action would explain its observed effects upon galactose transport.

One might expect arsenate to cause a stronger inhibition of transport in wild type cells in view of its action in mutants, each of which lacks one of the two pathways postulated for energy coupling. However, in cells exhibiting normal glycolysis and oxidative phosphorylation, the presence of more than one site for arsenate action could explain a reduction in the over-all effectiveness of this inhibitor, especially since the standard assay medium is high in phosphate. Although not shown here, in a phosphate-free medium 30 mM arsenate does inhibit the initial rate of galactose uptake by 85% and prevents the stimulation by lactate in wild type cells (W3902-). As discussed earlier (see introduction) other studies of active transport in E. coli have also led to the conclusion that energy can be derived either from respiration or from glycolysis (24–4). The present findings further show that, at least in the case of galactose, the link between electron flow and sugar transport is not an immediate one; respiration is not sufficient for galactose accumulation, but ATP need not be generated. Hong and Kaback (15) arrived at a similar conclusion in their studies of amino acid uptake in electron transfer coupling mutants.

The results do not give any information about the nature of the high energy intermediate which serves as the direct source of transport energy. In terms of current hypotheses for oxidative phosphorylation the observations are consistent with and cannot distinguish between the generation of a proton or electrochemical gradient, a high energy compound, or an energized state of the bacterial membrane.

The scheme for energy coupling presented here bears close analogy to the one postulated for the energy-dependent reduction of NADP by NADH in E. coli membranes. The direct energy source for this transhydrogenase activity is most likely a high energy intermediate which can be dissipated by uncouplers and which can be generated by either of the two pathways, respiratory chain oxidation of metabolites or ATP hydrolysis in the presence of a functional Mg$^{2+}$-, Ca$^{2+}$-stimulated ATPase (30–33).

In a previous paper (21) we presented evidence suggesting that energy coupling for the β-methylgalactoside transport system occurs during the entry rather than the exit of galactose: (a) the inhibition of galactose uptake by energy poisons was too great to be accounted for by an increased rate of sugar efflux; (b) no countercflow could be demonstrated; and (c) sodium azide did not change the $K_m$ for the exit of galactose induced by glucose. The effects of n-lactate reported here also support this conclusion. n-Lactate was used as a representative respiratory substrate and was found to stimulate the initial rate of galactose uptake to
3-fold. In contrast, it did not induce a significant change in the rate of exit of galactose from preloaded cells either in the presence or absence of energy poisons. If energy coupling were to occur during the exit process, one would expect n-lactate to act by decreasing the exit of galactose rather than by increasing the entry. Consequently the present observations also point to the entry process as the site of energy coupling.

The present state of knowledge precludes characterization of the molecular mechanism by which energy coupling to transport is accomplished. The problems involved in studying such a multicomponent, membrane-localized system are precisely the ones that have caused elucidation of the mechanism of oxidative phosphorylation to be one of the most persistent and elusive problems in biochemistry.

Acknowledgments—We wish to thank Dr. Herman Kalckar for his generous hospitality and his encouragement during this work. We are grateful to Miss Lena Alberico for her expert technical assistance. We would also like to thank Dr. G. B. Cox of the John Curtin School of Medical Research, Australian National University, Canberra, for his generous gift of bacterial strains.

REFERENCES

1. Scarborough, G. A., Rumley, M. K. & Kennedy, E. P. (1968) *Proc. Nat. Acad. Sci. U. S. A.* **60**, 951-958
2. Schairer, H. U. & Haddock, B. A. (1972) *Biochem. Biophys. Res. Commun.* **48**, 544-551
3. Simon, R. D. & Shallenberger, M. K. (1972) *Proc. Nat. Acad. Sci. U. S. A.* **69**, 2663-2667
4. Klein, W. L. & Boyer, P. D. (1972) *J. Biol. Chem.* **247**, 7257-7265
5. Pavlasova, E. & Harold, F. M. (1969) *J. Bacteriol.* **98**, 195-204
6. Mitchell, P., ed (1970) *Twentieth Symposium of the Society for General Microbiology*, pp. 121-166, Cambridge University Press, London
7. West, I. C. (1970) *Biochem. Biophys. Res. Commun.* **41**, 655-661
8. West, I. C. & Mitchell, P. (1972) *Biochem. J.* **127**, 50P
9. Kaback, H. R. & Milner, L. S. (1970) *Proc. Nat. Acad. Sci. U. S. A.* **66**, 1008-1015
10. Barnes, E. M., Jr. & Kaback, H. R. (1970) *Proc. Nat. Acad. Sci. U. S. A.* **66**, 1190-1198
11. Barnes, E. M., Jr. & Kaback, H. R. (1971) *J. Biol. Chem.* **246**, 5518-5522
12. Kaback, H. R. & Barnes, E. M., Jr. (1971) *J. Biol. Chem.* **246**, 5523-5531
13. Konings, W. M., Barnes, E. M., Jr. & Kaback, H. R. (1971) *J. Biol. Chem.* **246**, 5587-5591
14. Kebar, G. K., Gordon, A. S. & Kaback, H. R. (1972) *J. Biol. Chem.* **247**, 291-297
15. Hong, J. S. & Kaback, H. R. (1972) *Proc. Nat. Acad. Sci. U. S. A.* **69**, 3338-3340
16. Prezioso, G., Hong, J. S., Kebar, G. K. & Kaback, H. R. (1973) *Arch. Biochem. Biophys.* **154**, 575-582
17. Taylor, A. L. (1970) *Bacteriol. Rev.* **34**, 155-175
18. Wu, H. C. P., Boos, W. & Kalckar, H. M. (1969) *J. Mol. Biol.* **41**, 109-120
19. Huttin, J. D., Cox, G. B. & Gibson, F. (1971) *Biochem. J.* **124**, 75-81
20. Cox, G. B., Young, I. G., McCann, L. M. & Gibson, F. (1969) *J. Bacteriol.* **95**, 450-456
21. Barnes, J. R. & Boos, W. (1973) *J. Biol. Chem.* **248**, 4436-4445
22. Leive, L. (1965) *Proc. Nat. Acad. Sci. U. S. A.* **53**, 745-750
23. Leive, L. (1968) *J. Biol. Chem.* **243**, 2373-2380
24. Nett, H. C. & Heppel, I. A. (1963) *J. Biol. Chem.* **238**, 3685-3692
25. Lengeler, J., Hermann, K. O., Unsöld, H. J. & Boos, W. (1971) *Eur. J. Biochem.* **19**, 457-470
26. Huttin, G. & Kornberg, A. (1966) *J. Biol. Chem.* **241**, 5419-5427
27. Knapp, H., Boquet, P. L. & Röschenthaler, R. (1972) *Fed. Eur. Biochem. Soc. Lett.* **19**, 311-314
28. Chapman, A. G., Fall, L. & Atkinson, D. E. (1971) *J. Bacteriol.* **108**, 1072-1086
29. Boyen, P. D. (1968) in *Biological Oxidations* (Singh, T. P., ed) pp. 191-295, Interscience Publishers, New York
30. Fisher, R. J. & Sanadi, D. R. (1971) *Biochim. Biophys. Acta* **246**, 34-41
31. Coxe, G. B., Newton, N. A., Butlin, J. D. & Gibson, F. (1971) *Biochim. Biophys. Acta* **229**, 489-483
32. Harold, F. M. (1972) *Bacteriol. Rev.* **36**, 172-230
33. Gutnick, D. L., Kanner, B. I. & Postma, P. W. (1972) *Biochim. Biophys. Acta* **298**, 217-222
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*J. Biol. Chem.* 1973, 248:4429-4435.

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