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Carbohydrate Transport in Bacteria

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The Infinity of various beings is abiding in the one, and is an evolution from the one.

The Bhagavad Gita

"Beauty is truth, truth beauty,"—that is all Ye know on earth, and all ye need to know.

John Keats

INTRODUCTION

Nutrient acquisition is an important function of all living organisms, and many cellular activities are directed toward this goal. These activities include cellular locomotion, chemotaxis, and secretion of digestive enzymes. An integral aspect of the acquisition process is the transport of nutrients from the external environment through the cell barrier into the cytoplasm, where metabolism occurs. The cytoplasmic membrane, which serves as the cell barrier, not only allows selective uptake of desired nutrients, but also permits excretion of waste products. Much research has been directed toward determination of the molecular mechanisms by which hydrophilic compounds are transported through this hydrophobic cell barrier. Bacteria have been used widely in these studies, partly because they can be readily subjected to experimental manipulation. Large homogeneous populations of bacterial cells can be obtained easily, but perhaps the greatest advantage of working with these organisms is the fact that genetic manipulative techniques are well developed. Some of the transport systems studied in greatest detail are found in *Escherichia coli*, *Salmonella typhimurium*, and *Bacillus subtilis* for this very reason. Just as the universality of deoxyribonucleic acid, ribonucleic acid, and protein biosynthetic mechanisms is being established in bacteria and eucaryotes, so are the similarities of nutrient transport mechanisms becoming apparent. A detailed discussion of the catalytic systems respon-

sible for the transmembrane translocation of carbohydrates in bacteria is presented here.

Bacteria have evolved a variety of mechanisms by which solutes are transported into and out of living cells. Particular solutes may be either in equilibrium across a biological membrane or concentrated on one side. There are five carrier-mediated carbohydrate transport mechanisms now known to occur in microorganisms. These are represented schematically in Fig. 1, where a specific example of each type of system found in *E. coli* is depicted. As discussed below, these systems can be classified conveniently according to the source of energy coupled to the transport process. Facilitated diffusion is a process not coupled to metabolic energy and is therefore not capable of accumulating a substrate against a concentration gradient. Glycerol crosses bacterial cell membranes by this mechanism. Other systems are grouped together as "active" or "concentrative" transport systems since they are energy coupled and accumulate substrates against concentration gradients. The ability of an active transport system to concentrate its substrates on one side of a membrane

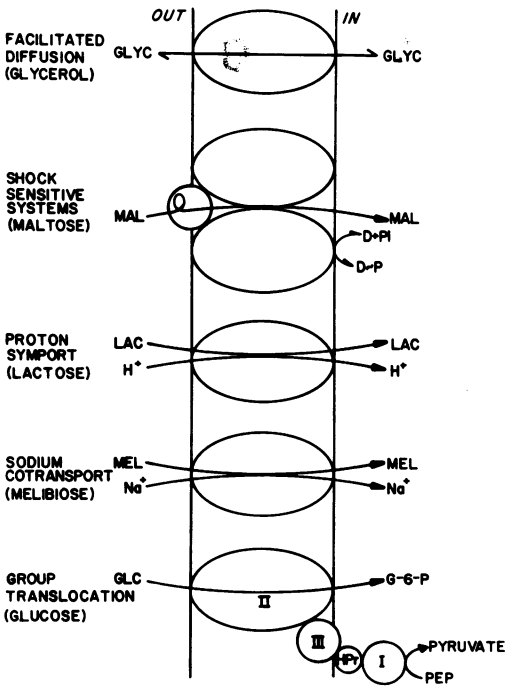

FIG. 1. *Carrier-mediated transport mechanisms. Representative examples (in parentheses) occur in E. coli. Abbreviations: GLYC, glycerol; MAL, maltose; LAC, lactose; MEL, melibiose; GLC, glucose; G-6-P, glucose 6-phosphate; PEP, phosphoenolpyruvate; D + Pi, nonphosphorylated donor plus inorganic phosphate; D ~ P, high-energy phosphorylated donor; I, enzyme I; II, enzyme II; III, enzyme III.*

must involve alteration of the carrier while the solute is passing from one side of the membrane to the other. The force driving this alteration is derived from specific products of cellular metabolism, and it is this source of metabolic energy that is used to classify the active transport systems. Examples of these types of systems occurring in *E. coli* and *S. typhimurium* include the maltose transport system, which is thought to utilize a chemical form of energy to concentrate its substrates, the lactose permease, which utilizes a proton electrochemical gradient as the source of energy for solute accumulation, and the melibiose permease, which functions by Na^+-sugar cotransport. Finally, glucose is a substrate of the phosphotransferase system (PTS), in which the substrate is phosphorylated during transport. Such a mechanism is referred to as group translocation. Detailed considerations of the nomenclature of transport phenomena have been presented elsewhere (148, 165).

Current experimental information concerning these different transport mechanisms is considered below. We discuss the mechanisms responsible for the regulation of carbohydrate uptake in bacteria, as well as the mechanisms by which the syntheses of certain transport systems are regulated. This review is meant to supplement and update previously published review articles concerned with these subjects (132, 152, 159, 180).

(The survey of literature for this review was completed in July 1979.)

FACILITATED DIFFUSION

Transport systems which catalyze facilitated diffusion aid in the rapid equilibration of a substrate across the cytoplasmic membrane, and because the process is not coupled to metabolic energy, the solute is not concentrated within the cell. Glycerol is the only carbohydrate known to be transported via facilitated diffusion in bacteria. Its uptake and dissimilation in *E. coli* has been well characterized by Lin (103). These results, which led to a demonstration of the glycerol facilitator, are described below.

Glycerol Transport System in *Escherichia coli*

It has been known for a long time that bacterial cells are not plasmolyzed by hypertonic solutions of glycerol. This suggests that glycerol is able to equilibrate rapidly across the cytoplasmic membrane. Whereas free diffusion may allow entry of glycerol, it was not known whether cells could effectively capture glycerol at low concentrations in amounts necessary for growth by using a passive mechanism. In 1965, Hayashi and Lin (58) showed that mutants of *E. coli* K-

12 lacking glycerokinase (the first enzyme involved in glycerol utilization) were not capable of accumulating [^{14}C]glycerol. Mutant strains possessing glycerokinase activity but lacking L-α-glycerophosphate dehydrogenase activity accumulated labeled L-α-glycerophosphate. The K_m of glycerokinase for its substrate and the K_m of intact cells for the uptake of glycerol were similar, suggesting that any accumulation of glycerol was due to trapping of glycerol by the action of glycerokinase.

Evidence for the facilitator protein was obtained by Sanno et al. (168), who examined the osmotic effects of glycerol on cells grown in the absence and presence of glycerol. The addition of a hypertonic solution of glycerol to cells grown on glucose resulted in plasmolysis, as demonstrated by a sharp increase in the optical density of the cell suspension; then the cells slowly regained their normal shape, as monitored by a decrease in the optical density of the cell suspension. Growth of the cells on glycerol greatly increased the rate at which they regained their shape. This suggested that a glycerol-specific facilitator was induced by growth on glycerol. Glycerol was not able to act as an inducer in glycerokinase-negative mutants, and in control experiments with NaCl as the permeant there was no difference in the rate of relief of plasmolysis between glucose- and glycerol-grown cells. Genes coding for the facilitator protein (*glpF*) and for glycerokinase (*glpK*) mapped at approximately 87 min on the *E. coli* chromosome (8, 17). Structural genes for other enzymes in the catabolic pathway were located in other regions of the chromosome, but all of these genes appeared to be regulated negatively by the product of the *glpR* gene, which mapped at approximately 74 min (30). The *glpR* protein is thought to combine with L-α-glycerophosphate, the true inducer, thereby causing induction of the *glp* regulon (58).

Facilitated Diffusion Systems in Other Bacteria

Facilitated diffusion systems for glycerol appear to be widespread. In addition to the systems in *E. coli* and *S. typhimurium*, glycerol facilitators occur in species of *Pseudomonas* (195), *Klebsiella* (168), *Shigella* (142), *Bacillus* (150), and *Nocardia* (19). In fact, in every case in which this transport mechanism has been studied, glycerol entry occurs by facilitated diffusion. Glycerol is fairly soluble in lipid bilayer membranes, and concentrative uptake would be wasteful due to rapid passive efflux. The facilitator thus serves to supply saturating amounts of glycerol to glycerokinase.

The only other report of a carbohydrate-facilitated diffusion system in bacteria was by Button et al. (18), who reported that maltose entry in *Staphylococcus aureus* was not coupled to phosphoenolpyruvate-dependent phosphorylation (group translocation) and concluded that uptake occurred by a diffusion process.

OSMOTIC SHOCK-SENSITIVE ACTIVE TRANSPORT

Shock-sensitive transport systems in gram-negative bacteria are so named because uptake via these systems is virtually abolished by the cold osmotic shock procedure of Neu and Heppel (123). The loss of uptake activity is due to a loss of the substrate binding proteins normally retained within the periplasmic space. All of the binding protein transport systems thus far described appear to utilize a chemical source of metabolic energy for intracellular solute accumulation. Many such systems for carbohydrates and amino acids have been discovered in *E. coli* and *S. typhimurium* (125, 180, 207) (Table 1). Maltose transport in *E. coli* is described below to illustrate the characteristics of shock-sensitive transport systems.

TABLE 1. *Shock-sensitive carbohydrate transport systems in E. coli*

System	Substrate(s)	Genetic locus	Map position (min)	Binding protein mol wt	Reference(s)
Arabinose	L-Arabinose, D-fucose, D-galactose, β-methyl-L-arabinoside, D-xylose, β-methyl-D-galactoside, lactose	*araE*	61	38,000	65, 173
Maltose	Maltose, maltotriose, and higher maltodextrins	*malB*	90	37,000	44, 77
Methyl-β-galactose	D-Glucose, D-galactose, β-glycerol-galactoside, L-arabinose, 6-deoxy-D-glucose, D-fucose, β-methylgalactoside, xylose	*mglA, mglB, mglC*	45	35,000	7, 180
Ribose	Ribose, ribulose	*rbsP*	83	30,000	204
Xylose	Xylose	*xylT*	78	ND[a]	178, 179

[a] ND, Not determined.

Maltose Transport System in *E. coli*

Cold osmotic shock of *E. coli* cells causes the release of the periplasmic maltose binding protein and destroys maltose uptake activity (77). For this reason transport of maltose and of other substrates of binding protein systems cannot be studied in membrane vesicles (51). Successful reconstitution of periplasmic binding proteins with membrane vesicles has been reported but not confirmed (207). Wilson suggested that the observed vesicular accumulation of radioactive substrates was due to ligand binding to the added binding proteins (207). Several reports have led to the suggestion that shock-sensitive transport systems are functional in vesicles in the absence of binding protein (180). However, most of the substrates referred to in these reports are transported by more than one permease system, and those substrates transported exclusively by binding protein systems were not accumulated in vesicles (77).

When binding proteins and their corresponding ligands are dialyzed against large volumes of buffer, a characteristic retention phenomenon is observed. The rates of disappearance of the ligands from dialysis bags containing the binding proteins are retarded relative to bags lacking the binding proteins. The loss of a ligand and its retention by the corresponding binding protein follow the mass law for retention phenomena, for which the following equation has been derived (180):

$$\frac{d[L]}{dt} = \frac{[L]\alpha}{1 + [P]/K_d}$$

where dL/dt is the rate of loss of ligand into ligand-free medium, $[P]$ is the binding site concentration, $[L]$ is the ligand concentration, K_d is the dissociation constant, and α is a constant determined by the diffusion properties of the ligand molecule and the experimental conditions, such as dialysis membrane porosity. This equation is valid only when the total ligand concentration is low in comparison with the total concentration of binding sites. When the ligand concentration is high compared with the binding site concentration, a loss of ligand from the dialysis bag reflects simple diffusion rates.

The mass law for retention phenomena has been verified with homogeneous maltose binding protein from *E. coli* (181). Maltose binding protein is a single polypeptide chain with a molecular weight of 37,000. In induced cells it comprises approximately 0.3% of the total protein. Maltose binding by the purified protein is heterogeneous. One type of binding had a K_d of 1.5×10^{-6} M with 0.5 mol of maltose bound per mol of binding protein, whereas another type had a K_d of 1×10^{-5} M with the same molar binding ratio. An explanation for this apparent heterogeneity is not yet available.

In biological situations where the concentration of a binding protein is high (as it is in the periplasm), the retention phenomenon may be of physiological significance. Thus, the entrance of a ligand into the periplasmic space may be 10^4 to 10^5 times faster than its exit. Ligand retention effectively raises the local concentration of the ligand so that the maximal rate of transport across the cytoplasmic membrane is attained. Periplasmic binding proteins may therefore function as K_m factors. This simple suggestion alone does not appear to explain the biological function of the maltose and other binding proteins for the following reasons: (i) maltose uptake does not occur in membrane vesicles; (ii) mutants that have specifically lost the maltose binding protein cannot utilize maltose (77); and (iii) maltose binding protein and other binding proteins are known to be necessary for chemotaxis toward their specific ligands (2). These and other observations have led to the proposal that it is the binding protein-ligand complex that interacts with the cytoplasmic membrane transport proteins and that this interaction leads to translocation of the substrate (180). No experimental evidence for or against this hypothesis has yet been reported, and the role of the binding protein in the translocation process remains uncertain.

The transport system for maltose and the larger maltodextrins is coded for by five genes within the *malB* region of the *E. coli* chromosome (64). Three of the five gene products have been identified. First, the *malE* gene is the structural gene for the periplasmic maltose binding protein (77). Second, the product of the *lamB* gene is an outer membrane porin that facilitates translocation of maltodextrins across the outer membrane (137, 188, 189). Third, the product of the *malF* gene is a cytoplasmic membrane protein. This fact was established by purification of the product of a *malF-lacZ* gene fusion. Antibodies prepared against the product of this gene fusion precipitated a protein that was found in the Triton X-100-soluble fraction of the wild-type cell envelope. This protein had a molecular weight of 50,000 on sodium dodecyl sulfate gels and was absent from strains which carried either nonsense mutations or *Mu* insertions within the *malF* gene (H. A. Shuman, T. J. Silhavy, and J. Beckwith, personal communication). The products of the other two genes within the *malB* regulon (*malK* and *malG*) have not yet been identified.

The *malB* regulon lies at approximately 90 min on the *E. coli* chromosome (8). Both the

maltose binding protein and maltose transport activity are induced more than 10-fold by growth on maltose, and both are repressed by mutations in the *malT* gene, which maps within the *malA* region at 74 min and is a regulatory gene both for the *malPQ* operon (which codes for the maltose catabolic enzymes) and for the two operons of the *malB* regulon (8).

Shock-sensitive transport systems are considered to be primary active transport systems because chemical energy is directly coupled to the uptake process. Similar energy-coupling characteristics have been observed for all shock-sensitive transport systems thus far investigated (14, 15, 31, 83, 205), and a discussion of energy coupling to maltose transport is presented below to introduce these characteristics.

The following observations concerning energy coupling to maltose transport have been reported (44). (i) Uncouplers of oxidative phosphorylation (dinitrophenol, azide, and carbonyl cyanide-*m*-chlorophenyl hydrazone) only partially inhibited maltose accumulation, and fructose (a glycolytic energy source) stimulated maltose uptake much more than did D-lactate in an adenosine triphosphatase (ATPase)-negative mutant. (ii) Maltose uptake was sensitive to arsenate inhibition (44), although other binding protein systems appear to be more sensitive (126).

Oxidative phosphorylation inhibitors act by collapsing proton and/or ion gradients that normally exist across the cytoplasmic membrane. Therefore, it is not surprising that these inhibitors virtually destroy solute transport coupled to a chemiosmotic force, as described below. It is also not surprising that these inhibitors reduce uptake via binding protein systems since they inhibit oxidative phosphorylation, thus lowering adenosine 5'-triphosphate (ATP) pools. However, since ATP pools can also be supplied by substrate-level phosphorylation, primary active transport can still occur. In mutants lacking ATPase, the effect of oxidative phosphorylation uncouplers on primary active transport is greatly reduced compared with the effect in wild-type cells (44). In these mutant strains most of the ATP pool arises from substrate-level phosphorylation, and the dependence on oxidative phosphorylation for ATP is eliminated. These considerations explain why fructose is a much better energy source for maltose uptake than is D-lactate in mutants lacking ATPase activity. When metabolized through glycolysis, fructose yields chemical energy via substrate-level phosphorylation, whereas D-lactate metabolism produces ATP mainly through oxidative phosphorylation. Generally, binding protein systems are very sensitive to arsenate inhibition (126) because this inhibitor greatly reduces the ATP pool (82). Maltose uptake is not as strongly inhibited by arsenate as several other systems (44) for unexplained reasons. It is possible that the maltose uptake system has a higher affinity for the chemical energy source than do the other systems studied. Proton symport has been shown not to occur during operation of the binding protein-dependent β-methylgalactoside transport system (60). These observations are consistent with the hypothesis that a metabolite, such as ATP, rather than a chemiosmotic source of energy drives active transport via binding protein-dependent systems, such as the one for maltose.

Two observations have been made which are not easily explained by the energy-coupling hypothesis presented above. First, colicin K abolished the chemiosmotic force in an ATPase-negative mutant strain of *E. coli* without lowering ATP levels, but shock-sensitive transport systems were inhibited (130). Second, temperature-sensitive mutants of *E. coli* have been isolated which exhibited energy-coupling defects for both the primary and secondary active transport systems even when the ATP pool was elevated (100, 101). These results led to the hypothesis that binding protein-dependent transport is not directly coupled to chemical energy but is energized indirectly through an energy-transducing component common to all active transport systems (101).

It is interesting that maltose efflux from *E. coli* cells does not appear to be mediated by the same system that catalyzes influx (44). Maltose efflux is sensitive to inhibition by uncouplers of oxidative phosphorylation and is relatively insensitive to arsenate inhibition. Chemiosmotic energy may function to keep the maltose carrier in the proper orientation for maltose efflux or may be required for efflux in the downhill direction for other reasons. It is not known whether the proteins that catalyze maltose efflux are encoded by genes in the *malB* region (44). However, mutations in the structural genes coding for components of the β-methylgalactoside binding system do not affect exit of this galactoside (183, 206).

Hong et al. (67) have recently presented evidence implicating acetyl phosphate as the immediate energy source for binding protein-dependent transport systems. By limiting the synthesis of this compound using genetic manipulations and metabolic inhibitors, these workers showed that *E. coli* cells were not able to take up binding protein system substrates unless a pathway for acetyl phosphate synthesis was provided. However, under the experimental conditions used, ATP levels were reduced in order to limit acetyl phosphate production. Thus, it is

still possible that some other high-energy compound whose synthesis is dependent upon ATP may be a required energy source for these transport systems. A direct effect of exogenous acetyl phosphate on binding protein-dependent transport has not been demonstrated.

Other Shock-Sensitive Systems

Several shock-sensitive carbohydrate transport systems have been detected in *E. coli*. These are listed in Table 1. Most of the information presented was obtained from two recent reviews (180, 208). It is interesting to note that most of the carbohydrates listed are able to enter the cell via transport systems which are coupled to other sources of metabolic energy. This may offer the cell survival advantages under a variety of environmental conditions.

PROTON-LINKED ACTIVE TRANSPORT

Principles of Chemiosmotic Coupling

In 1961, Mitchell proposed the chemiosmotic coupling hypothesis for conservation of energy across biomembranes (116). In this hypothesis he suggested that energy derived from biological oxidations could be stored across a membrane in the form of a proton electrochemical potential difference. It has since been shown that this potential is the energy source used to drive several membrane-related cellular processes, including oxidative phosphorylation (110, 209) and nutrient transport (117, 210). Since its conception, the chemiosmotic coupling hypothesis has revolutionized the study of bioenergetic coupling mechanisms, and for his work Mitchell was awarded the Nobel Prize in Chemistry in 1978. Mitchell's accolades are well deserved, for he not only recognized the possibility of this energy-coupling pathway but also provided much of the supportive experimental evidence. To be fair, however, it must be noted that his proposals were not without precedent in the literature. In 1945, Lundegardh proposed that if the cytochrome system was arranged anisotropically across a membrane, substrate oxidation would result in the production of H^+ on one side and the consumption of H^+ on the other (107). This hypothesis led to the formulation of the primary postulate of the chemiosmotic coupling model. It explained how energy released from the electron transport system could be converted to an electrical potential created by a proton concentration difference across a membrane. To complete the coupling process, the return flow of protons across the membrane could be used to drive an energetically unfavorable process, such as ATP synthesis or the accumulation of a substrate against a concentration gradient. Many reviews concerning the existence and function of chemiosmotic coupling mechanisms have appeared (53, 55, 90, 119, 120, 148).

According to the hypothesis of Mitchell, the immediate result of biological oxidations via the electron transport system is the expulsion of protons from the cell. This process generates a chemical gradient of hydrogen ions, which can be detected as a pH difference between the aqueous phases on the two sides of the membrane and as an electric potential difference across the membrane. The sum of these potentials is expressed by the following equation:

$$\Delta\mu_{H^+} = F \cdot \Delta\Psi - 2.3RT \cdot \Delta pH$$

where $\Delta\mu_{H^+}$ is the total chemiosmotic or proton motive force (PMF), F is the Faraday constant, $\Delta\Psi$ is the electric potential across the membrane, R is the gas constant, T is the temperature in degrees Kelvin, and ΔpH is the proton chemical gradient. This equation can be rearranged to form an equation similar to the one describing the electron motive force of an electrochemical cell as follows:

$$\frac{\Delta\mu_{H^+}}{F} = \Delta\Psi - \frac{2.3RT \cdot \Delta pH}{F} = \Delta\Psi - Z \cdot \Delta pH$$

where Z is a combination of constants (2.3 RT/F) which at 37°C has a value of approximately 60 mV/pH unit. Therefore, the energy potential or PMF across a biological membrane is conceptually similar to the electron motive force of a battery. Several "proton pumps" within biological systems have now been identified. These include the respiratory chain of bacteria and mitochondria (55), light-sensitive bacteriorhodopsin (97), light-sensitive electron transfer in chloroplasts and photosynthetic bacteria (119), and the membrane-bound Ca^{2+}-Mg^{2+} ATPase of bacteria, mitochondria, and chloroplasts (55, 90). The diversity of the proton pump mechanisms in both procaryotic and eucaryotic organisms is an indication of the importance and universality of chemiosmotic energy coupling.

In order for a proton gradient to exist, the membrane must be impermeable to both hydrogen and hydroxyl ions. In 1967, Mitchell and Moyle demonstrated that membranes are impermeable to these ions by measuring the rate of hydrogen ion titrations across mitochondrial membranes (121, 122). Proton impermeability is now known to be a characteristic of many biological membranes and of artificial phospholipid bilayers (55).

Chemiosmotically coupled transport has been defined as secondary active transport. The primary transport event, which provides energy for secondary active transport, is proton extrusion

(the conversion of light, electrical energy, or chemical energy into electroosmotic energy). The coupling of this energy source to the influx of a compound (for example, lactose) occurs so that an endergonic process (the accumulation of a nutrient in the cell) can be coupled to an exergonic process (protons moving down a concentration gradient).

Mitchell has proposed the following three terms to define the mechanisms by which metabolites are linked to ion transport: symport, antiport, and uniport. The term symport describes a system in which two substrates are transported in the same direction by a carrier. Antiport systems are those in which two substrates are translocated in opposite directions. Uniport is defined as a process in which a solute moves across a membrane but is not tightly coupled to the movement of another species. For further discussion and clarification of these definitions, see Rosen and Kashket (148).

An involvement of the membrane potential in energizing active transport is now almost universally accepted. However, the actual coupling mechanism is not yet understood, and there is still some controversy as to the immediate source of energy used to drive active transport. Hong and co-workers (66, 102, 194) have isolated three separate *E. coli* mutants that display energy-coupling defects in PMF-driven active transport systems. All three mutations give rise to temperature-sensitive phenotypes and map within the *ecf* gene at 64 min on the revised *E. coli* chromosomal map. These mutants are able to grow normally on glucose, succinate, fumarate, malate, or D-lactate at 25°C but are not able to grow on these compounds or on nutrient broth at 42°C. By assaying energy production (either membrane potential or ATP synthesis), substrate transport, and membrane proton permeability, these mutants have been shown to differ with respect to their energy-coupling characteristics. One mutant could not efficiently maintain a membrane potential at the nonpermissive temperature. The loss of the ability to maintain the potential was not due to greater membrane proton permeability and was not repaired by addition of dicyclohexylcarbodiimide (102). It was shown that this mutant could briefly form a membrane potential upon addition of exogenous energy sources and that this potential stimulated substrate uptake. However, its inability to maintain a potential difference resulted in a loss of transport activity and oxidative phosphorylation. Consequently, the mutant was not able to grow at the nonpermissive temperature.

The second mutant (66) was able to maintain a membrane potential but was not able to couple this energy to active transport at the nonpermissive temperature. The third mutant (194), like the first, was not able to maintain the PMF but, unlike the first, exhibited greater membrane proton permeability. These studies suggested the possibility that the energy-coupling factor (ECF) protein functions in coupling the PMF to active transport. Hong (66) has suggested two models to explain the role of the ECF protein in the transport process. In the first model the ECF protein rather than the substrate permease acts as the proton symporter. In the second model the ECF protein serves as the direct driving force for transport by first becoming energized by the PMF. The second model is thus reminiscent of the conformational coupling hypothesis. Experimental support for either of these models and information concerning the biological function of the ECF protein await further research.

Lactose-Proton Symport

The lactose permease in *E. coli* has the distinction of being the first bacterial uptake system to have been described (23). It was further recognized in 1956 that the transport protein was coded for by a specific gene within the *lac* operon, the *lacY* gene, which was distinct from the *lacZ* gene, which codes for β-galactosidase (143). Mitchell (118, 120) first suggested that lactose accumulation was coupled to chemical energy via proton symport, and the initial experimental support for this hypothesis came from his laboratory (201, 202; I. C. West and P. Mitchell, Biochem. J. **127**:56P, 1972). Mitchell and West demonstrated that the addition of thiomethyl-β-D-galactopyranoside (TMG) (a non-metabolizable sugar analog transported by the lactose permease) to energy-depleted cells caused proton influx against an electrochemical gradient, thereby acidifying the cytoplasmic compartment with concomitant alkalinization of the external medium. Their results have been confirmed by recent experiments of Collins et al. (24) and clearly illustrate the coupled movement (symport) of TMG and protons.

Since TMG gradients could drive proton entry, the converse should also be true; i.e., proton (or electrochemical) gradients should successfully drive TMG accumulation. Indeed, this is the essence of the chemiosmotic coupling hypothesis. The ability of an artificially produced inwardly directed PMF to drive TMG uptake has been reported (46, 210). Either a membrane potential created by a potassium ion gradient in the presence of valinomycin or a pH gradient produced by the addition of acid to energy-depleted cells stimulated TMG entry.

Within the past 10 years, there has been a great deal of research with whole cells which

supports the chemiosmotic coupling hypothesis. More recently, important experimental support for this energy transducing model has come from studies with membrane vesicles of *E. coli*. Kaback and co-workers have shown that vesicles are capable of producing a PMF when supplied with an appropriate energy source, such as D-lactate or reduced phenazine methosulfate (134, 174). Furthermore, production of the PMF is mandatory for the active accumulation of substrates, such as lactose (135, 174). From this work and earlier studies with whole cells, involvement of the PMF in the active accumulation of TMG (or lactose) is well established, as is proton symport. However, a number of controversies concerning the action of the lactose permease remain to be resolved. These include the stoichiometry of proton and lactose entry, the number of proteins and binding sites involved in the permeation process, the actual translocation mechanism, and the involvement of the PMF in the initial binding of the substrate. These subjects are discussed below.

West and Mitchell (202) were the first to study the problem of stoichiometric proton and sugar movement. By simultaneously measuring hydrogen ion uptake and lactose uptake in energy-depleted *E. coli* cells, they arrived at a stoichiometry of unity. More refined experiments have been directed toward determining a correlation between the total PMF and the chemical potential of sugar. Assuming an entry of protons and sugar molecules at a ratio of 1:1 and assuming that leakage is negligible, then the electrochemical potential of H^+ should be in equilibrium with the chemical potential of sugar, as expressed by the following relationships (71):

$$\Delta\mu_{H^+} = \Delta\Psi - Z\cdot\Delta pH$$

$$\Delta\mu_{sugar} = -Z\cdot\log\left[\frac{\text{sugar (in)}}{\text{sugar (out)}}\right]$$

$$\text{At equilibrium, } \Delta\mu_{H^+} = \Delta\mu_{sugar}$$

Through simultaneous measurements of both $\Delta\mu_{H^+}$ and $\Delta\mu_{TMG}$ the ratio of 1:1 has been verified (46, 135). However, Maloney and Wilson (111) reported data which suggested that various substrates of the lactose permease have different proton-substrate entry ratios, and Ramos and Kaback suggested that the ratio of lactose entry to proton entry into membrane vesicles varied with the external pH (135, 136). More recently, Zilberstein et al. (219) and Hamilton (H. A. Hamilton, personal communication) independently arrived at a ratio of 1:1 and showed that this ratio was independent of pH. Thus, the early studies of West and Mitchell have been verified.

The lactose transport system appears to consist of a single protein encoded by the *lacY* gene. However, purification of this protein and reconstitution of lactose transport in membrane liposomes has not yet been accomplished (180). Toward this end, Belaich et al. (12) solubilized a protein from *E. coli* membranes that exhibited thiodigalactoside (TDG) binding properties. These investigators used nonionic detergents in the hope that the M protein would be denatured to a minimal extent so that further work could be directed toward preparation of the homogeneous protein. Altendorf et al. (4) used 5 to 6 M aprotic solvents, such as 90% hexamethylphosphoric triamide, to solubilize 50 to 80% of the total protein of membrane vesicles capable of transporting lactose. After separation of the solvent from the membrane proteins by ultracentrifugation, the active fraction was incorporated into vesicles of *E. coli* ML35, a lactose transport-negative mutant, and lactose uptake was detected after the addition of an energy source, such as D-lactate.

Recently, Villarejo and Ping (199) found that the M protein became randomly distributed between the outer and inner membranes during the separation of these membranes by several methods. They were able to detect the M protein in sodium dodecyl sulfate gels and observed a molecular weight similar to previously published values. Their observation of the randomization of this protein will be an important consideration in future attempts at its isolation. It is hoped that these advances will lead to the purification and reconstitution of the lactose transport system in the near future.

On the basis of transport inactivation and competitive binding studies, it has been suggested that there are two distinct sugar binding sites on the lactose permease. In 1965, Fox and Kennedy (48) reported that *o*-nitrophenyl-β-D-galactopyranoside uptake via the lactose permease was blocked by the addition of the sulfhydryl reagent *N*-ethylmaleimide (NEM) and, furthermore, that NEM inactivation could be prevented by prior addition of TDG. The affected component was found to be the membrane-bound lactose permease (47). Surprisingly, a number of other lactose permease substrates, including lactose, failed to protect the protein from NEM inactivation. On the basis of these studies and others, the lactose permease substrates were divided into two categories, namely, those that could protect against NEM inactivation and those that could not (20, 78). Table 2 lists some of the members of these classes, along with some of the apparent transport K_m values in *E. coli* ML strains.

Class I substrates (Table 2) were not able to

TABLE 2. *Lactose permease substrate classes*[a]

Class	Substrate
I	Lactose
	o-Nitrophenyl-β-D-galactopyranoside
	Thiomethyl-β-D-galactopyranoside
	Isopropyl-β-D-thiogalactopyranoside
II	Thiodigalactoside
	Melibiose
	Phenyl-β-D-galactoside
	Glycerol-β-D-galactoside
	Allolactose

[a] Assignment of substrate to class is based on work reported in references 20, 78, 80, and 217.

protect against NEM inactivation, whereas members of class II could. Melibiose and TDG were the most effective protecting agents. Binding studies conducted in the laboratory of Kennedy in which various sugars were tested for their ability to displace M protein-bound [^{3}H]-TDG also distinguished the two classes of sugars (79). In general, it was found that members of class II, such as melibiose, could displace TDG, whereas members of class I had at best a minor effect. It was also found that class II substrates inhibited binding of class I substrates, but the converse did not occur; i.e., class I substrates did not inhibit class II substrate binding (79). These studies were interpreted in terms of a model involving two binding sites on the permease protein, with a cysteine group near one of the sites to allow substrate bond protection against NEM inactivation.

Kepes (81) has argued that greatly varying affinities between the two classes of lactose permease substrates could account for the competitive inhibition and NEM protection effects when substrates have been compared at the same concentration. Carter et al. (20) also considered the possibility that class I sugars bind to the same site as class II sugars but that their stereochemistry does not protect the reactive cysteine. At concentrations 10-fold higher than the TDG concentration, neither *o*-nitrophenyl-β-D-galactopyranoside nor lactose significantly reduced the protective effect of TDG. These authors also estimated that phenyl-β-galactoside, allolactose, and glycerol-β-galactoside had apparent kinetic affinity constants in the millimolar range, which is greater than would be expected for a single-site model based on differing affinities.

Wright et al. recently reexamined the two-site hypothesis by using *E. coli* cytoplasmic membranes which contained levels of the *lacY* carrier about 10-fold higher than the level in wild-type cells (218). Sonication followed by sucrose density gradient centrifugation was employed to prepare cytoplasmic membranes free of outer membranes. These preparations were found to bind class I compounds (lactose, isopropyl-β-thiogalactoside, and nitrophenyl-β-galactoside) with low affinity (K_d, 5 to 15 mM) but class II compounds (nitrophenyl-α-galactoside, TDG, and dansyl-β-galactoside) with high affinity (K_d, 0.02 to 0.05 mM). Low-affinity class II compounds such as glycerol-β-D-galactoside and allolactose were not examined. When a class I compound such as lactose was employed at a high concentration (0.9 M), protection from NEM was observed. Thus, although the data may not be sufficient to warrant generalization, these results were consistent with the conclusion that all substrates bind to a single site on the permease, thereby protecting the essential cysteine residue from derivatization by NEM.

Several genetic studies also appear to be consistent with a single-site model. Langridge (96) examined more than 1,000 *lacY* mutants and found none that transported only class I or class II substrates. He concluded that there must be some other explanation for the NEM protection pattern than the two-site model. Hobson et al. (63) also used high-resolution genetic techniques and failed to isolate mutants that would support the two-site hypothesis. Finally, Fried (49) reported the isolation of a mutant (presumably with a mutation in the *lacY* gene) in which the genetic lesion partially abolished facilitated diffusion of lactose. The residual uptake activity was not affected by either TDG or sulfhydryl group inhibitors. It is conceivable that the lactose permease in this mutant lacks the class II binding site but retains the class I site.

An interesting observation, which may be relevant to the original two-site hypothesis of Fox and Kennedy, is that whereas class I substrates are accumulated against large concentration gradients, many of the class II compounds which have been tested are accumulated poorly (218). Thus, substrates which protect against NEM inactivation may enter the cell by facilitated diffusion (uniport), whereas those that do not protect appear to cross the membrane by secondary active transport (proton symport). These observations suggest that substrate binding to a single active site on the permease protein can induce either of two conformations, one of which requires proton binding and translocation for transmembrane solute transport and the other of which does not. Only in the former conformation (induced by class I substrates) would the active cysteinyl residue be exposed to NEM. According to this interpretation, the sulfhydryl group would be buried (not accessible) when a class II substrate is bound to the single active site of the permease protein.

Relevant to these suggestions are studies with

the glucose enzyme II of the PTS. This protein catalyzes glucose uptake exclusively by group translocation (see below), but galactose has been reported to cross the membrane via this enzyme II by facilitated diffusion (87). In this example, as well as in that of the lactose permease, it is possible that binding of an "abnormal" substrate analog to a permease protein can uncouple solute translocation from the energy-coupling mechanism. Facilitated diffusion of the analog is the necessary consequence of energy-uncoupled transport.

Mechanism of Energization of Lactose Uptake

As stated above, the actual mechanism of energization of active transport by the PMF is still unknown. Cecchini and Koch (21) studied the effects of the proton uncouplers carbonyl cyanide-*m*-chlorophenyl hydrazone and carbonylcyanide-*p*-trifluoromethoxyphenyl hydrazone on the uptake of a chromogenic substrate of the lactose permease, *o*-nitrophenyl-β-D-galactopyranoside. Experiments were conducted with both energy-proficient cells and cells depleted of energy reserves by treatment with methyl-α-D-glucopyranoside and sodium azide. Transport rates were determined by rates of *o*-nitrophenyl-β-D-galactopyranoside hydrolysis. Uncoupler effects on energy-proficient cells were as expected in that 50 to 60% of the control activity was lost. However, energy-depleted cells not preloaded with a lactose permease substrate were not able to take up *o*-nitrophenyl-β-D-galactopyranoside unless a proton uncoupler was added. The authors concluded that energy-depleted cells were not able to catalyze the net movement of charge (in this case, protons) across the membrane. The addition of the uncoupler presumably allowed dissipation of the potential created by the concomitant influx of protons and sugar molecules, thereby allowing diffusion of the sugar down a concentration gradient.

In 1978, Wilson (207) presented a model in which he suggested that the lactose permease can exist in deenergized cells in the following two states: a saturable high-affinity form and a nonsaturable low-affinity form. He further hypothesized that approximately 20% of the deenergized carrier is in the high-affinity form and that the function of the PMF is to convert the permease to a form with low affinity on the inside and high affinity on the outside.

Lancaster and Hinkle (95) investigated lactose-facilitated diffusion and active transport catalyzed by membrane vesicles of *E. coli*. They found that reversal of the electrochemical proton gradient reversed the direction of transport. The apparent K_m for influx by facilitated diffusion was the same in both right-side-out and wrong-side-out vesicles. Therefore, they concluded that the translocator was intrinsically symmetrical with respect to the mechanism of translocation.

In earlier reports (209, 217), it had been suggested that the function of transport energy was to convert substrate exit from a saturable reaction to a nonsaturable first-order reaction. Therefore, energy did not directly affect substrate uptake. This proposal was apparently confirmed by the finding that the V_{max} values of lactose entry into vesicles and of the *o*-nitrophenyl-β-D-galactopyranoside hydrolysis by whole cells described above were increased by energy (21, 94). A lactose transport energy-uncoupled mutant was isolated which exhibited elevated lactose permease activity yet was defective in TMG accumulation. This mutant was much more sensitive to inhibition by sulfhydryl reagents and had a much greater lactose efflux rate than did the parental strain. These observations can be interpreted in terms of the theory that energy functions to lower substrate binding affinity on one side of the membrane (211, 212).

Another phenomenon which has been observed by several investigators is the ability of accumulated substrate on one side of a membrane to stimulate transport of that substrate on the opposite side of the membrane (13, 84, 144). This coupling process occurs in the absence of active transport (i.e., in the presence of energy poisons) and produces a transient accumulation of radioactively labeled extracellular substrate due to efflux of intracellular substrate. At saturating external substrate concentrations and low pH, the flux coupling ratio (ratio of substrate to protons transported) approaches 1:1. It is not clear why this ratio is less than 1:1 under certain conditions, and such a value raises a question as to whether there is a proton leak, a breach in obligate proton symport, or substrate-independent efflux. The addition of proton conductors caused only a partial inhibition of this counterflow phenomenon. Since uncouplers would only prevent the portion of transient accumulation due to effluxed protons coupled to sugar influx, it is possible that a significant portion of the overshoot is due to direct sugar exchange from inside to outside, independent of the PMF produced by exiting substrate.

In reports from the laboratory of Kaback (149, 175), it has been suggested that binding of lactose permease substrates is greatly influenced by the PMF. This work was conducted with *E. coli* membrane vesicles and dansyl galactosides.

Accumulation of these sugar analogs was not detected, but preferential binding of the analog to membrane vesicles containing the lactose permease was observed. Kaback noted that in the presence of a utilizable energy source such as D-lactate, much more of the sugar analogs was bound, as detected by changes in fluorescence. These observations appear to support the high affinity-low affinity model described above. However, the conclusions of Kaback have been disputed in a recent report. Overath et al. (124) found that dansylated derivatives of lactose are indeed transported by vesicles, although slowly. These authors suggested that the changes in fluorescence observed by Kaback were due to a difference in the environment of the dansylated derivative on the inside of the vesicles. They further stated that the vesicular M protein was not present in a sufficiently high concentration to allow determination of differences between specific and nonspecific binding.

Kaczorowski et al. recently conducted detailed kinetic studies on lactose efflux from *E. coli* membrane vesicles (72, 73). Their results showed that unidirectional lactose efflux was highly dependent on the pH of the medium, occurring more rapidly at pH 7.5 than at pH 6.5 or 5.5. By contrast, counterflow with saturating concentrations of lactose on both sides of the membrane was much more rapid (about 10-fold) and was independent of the external pH. The unidirectional efflux (but not counterflow) was accompanied by the transient formation of a membrane potential (negative inside), as demonstrated by the accumulation of proline or of K^+ in the presence of valinomycin. This effect was blocked by proton-translocating uncouplers, such as carbonyl cyanide-*m*-chlorophenyl hydrazone. On the basis of these and other observations these authors concluded that the lactose permease functions by a carrier-type mechanism. In the undirectional efflux process, lactose and protons bind to vesicle interiors and are translocated as a neutral complex across the membrane. Lactose, followed by the proton, is then released into the external medium, and finally, translocation of the negatively charged (free) carrier back to the cytoplasmic surface of the membrane occurs. Proton release on the external surface and/or translocation of the free carrier back to the inner surface is presumed to be the rate-limiting step. In counterflow, it is postulated that deprotonation does not occur, but the neutral carrier cycles with rapid dissociation of the lactose-carrier complex at both the cytoplasmic and external surfaces. This presumption accounts for the pH independence and the rapid rate of exchange transport.

SODIUM-MELIBIOSE COTRANSPORT IN *E. COLI* AND *SALMONELLA TYPHIMURIUM*

In eucaryotic cells, the existence of sodium cotransport systems for sugars and amino acids has been known for many years (176). MacLeod and co-workers (37, 38, 185) were the first to provide evidence for an Na^+ cotransport system in bacteria by showing the marked dependence of the accumulation of α-aminoisobutyric acid on sodium ions by a marine pseudomonad. The only known carbohydrate-sodium cotransport system in bacteria was first described by Stock and Roseman in 1971 (187). These workers discovered that TMG, a non-metabolizable sugar analog, was transported by a sodium ion-dependent mechanism via the melibiose-induced transport system in *S. typhimurium*. Under the experimental conditions employed, the addition of sodium ions stimulated uptake of labeled TMG by more than 100-fold. This increase was reflected only in a decrease in the K_m value for TMG uptake; V_{max} was not affected. Lithium was the only other cation which stimulated TMG accumulation. Demonstration of simultaneous translocation of both sodium and TMG was complicated by the rapid efflux of sodium via the sodium-proton antiport pump. However, Stock and Roseman did detect a very rapid uptake of Na^+ ions in the presence of TMG, although Na^+ efflux prevented the cellular pool of this cation from more than briefly approaching its external concentration. The initial uptake data suggested that the entrance ratio of Na^+ to TMG was 1:1.

Tsuchiya et al. (196) have shown that the melibiose-induced transport system in *E. coli* also accumulates TMG via sodium cotransport. In some *E. coli* K-12 strains this system is peculiar in that its synthesis is temperature sensitive and is not induced at 37°C. Attempts to stimulate TMG uptake by creation of artificial pH gradients failed, indicating that TMG influx was not coupled to proton movement. Further experiments were performed, in which proton and sodium gradients were aligned in opposite directions. This was accomplished by diluting cells suspended in a pH 6.0 buffer into a buffer at pH 8.0 and simultaneously adding a proton ionophore, thus generating an intracellularly negative membrane potential produced by the rapid efflux of protons. Proton efflux provided a driving force for sodium ion accumulation. The sodium ion gradient was controlled by exposing cells with low intracellular sodium concentrations to high external sodium concentrations. TMG accumulation increased 17-fold compared

with controls to which no sodium was added. Furthermore, when the sodium chemical gradient or the proton gradient (necessary for Na^+ extrusion) was limited, TMG transport decreased. If both the pH and sodium gradients were eliminated, no sugar accumulated. Since the influx of Na^+ ions reduced the artificially imposed membrane potential and the Na^+ chemical gradient, the stimulation of sugar transport was only transient.

In another series of experiments, the external pH of the reaction mixture was monitored instead of TMG uptake. If TMG entered via proton symport, the external pH should have risen upon addition of the sugar analog. On the other hand, if TMG transport was coupled to Na^+ entry, the pH should have dropped due to the proton efflux stimulated by the decrease in membrane potential created by the sodium ion influx. The latter was found to be the case. Furthermore, measurement of the extracellular sodium concentration with a sodium-specific electrode showed an immediate decrease in this concentration upon addition of TMG (196). Evidence that only TMG permease II is responsible for Na^+-dependent TMG entry has been obtained genetically (196).

The melibiose-induced TMG-sodium cotransport system in *E. coli* has been characterized further by work with metabolic inhibitors and either whole cells or membrane vesicles (106). The effects of these inhibitors were as follows. The sulfhydryl group reagents NEM and *p*-chloromercuribenzoate were both potent inhibitors of TMG uptake, whereas iodoacetate, a glycolytic inhibitor, had little effect; proton ionophores eliminated TMG accumulation, and cyanide and azide, which independently caused little inhibition, together inhibited uptake more than 90%. In vesicles, the addition of the artificial electron donor pair ascorbate-phenazine methosulfate stimulated TMG uptake, but only in the presence of Na^+ or Li^+. These observations support the role of the proton gradient in creating the sodium ion chemical gradient necessary for TMG accumulation.

Tokuda and Kaback (192) studied the energy-coupling mechanism responsible for sodium-dependent TMG transport in membrane vesicles of *S. typhimurium*. Their studies were directed specifically toward (i) the relationship between the PMF and sodium-TMG cotransport and (ii) the mechanism by which intracellular sodium concentrations are maintained at low levels. When membrane vesicles were incubated with either D-lactate or reduced phenazine methosulfate, vesicles prepared from melibiose-induced cells catalyzed TMG accumulation in the presence of Li^+ or Na^+. Furthermore, these workers found that TMG stimulated $^{22}Na^+$ uptake, with equimolar amounts of the two compounds taken up. The source of energy used to form the chemical gradient of sodium ions was an artificially imposed membrane potential created by the imposition of a potassium gradient and the addition of valinomycin. Neither TMG nor sodium uptake occurred if either valinomycin or the potassium gradient was omitted. Silva and Dobrogosz (182) have also demonstrated that cotransport of sodium and TMG is associated with extrusion of protons by whole cells of *S. typhimurium*. Cells grown on lactate and not induced by melibiose showed no proton extrusion upon addition of TMG and sodium.

Van Thienen et al. (198) concluded that proton efflux was not directly linked to sodium-TMG cotransport in *S. typhimurium* cells but was probably indirectly regulated by metabolism induced by the uptake process. These conclusions were based on several observations. In energy-starved cells obtained by treatment with methyl-α-D-glucoside and sodium azide, sodium-TMG symport was not associated with proton efflux. These authors predicted that since influx of Na^+ had to be compensated for electrically by exit of a cation or entrance of a permeant anion, the passive ion permeability of the membrane might be increased in energy-depleted cells. This suggestion was substantiated by the observations that partially energy-depleted cells catalyzed proton efflux during sodium-TMG cotransport and that proton extrusion led to drastically reduced cellular ATP levels. Dicyclohexylcarbodiimide, an ATPase inhibitor, inhibited proton extrusion. These results indicated that the ATPase was at least partially responsible for proton extrusion.

Recently, Tokuda and Kaback (193) presented experimental data which suggested that membrane vesicles of melibiose-grown *S. typhimurium* cells displayed sodium-dependent binding of *p*-nitrophenyl-α-D-[6-^{3}H]galactopyranoside. They observed that the number of binding sites at saturation and the affinity of the transport system for its substrate increased with sodium and D-lactate additions. Similar increases were obtained by imposition of an artificial membrane potential in the presence of a high internal potassium concentration, valinomycin, and sodium. Through binding studies under conditions of varying pH and with the addition of various ionophores (including valinomycin, nigericin, and monensin), these workers found that binding varied with the electrochemical proton gradient and was particularly sensitive to the electrical component. Based on these findings,

they proposed that the PMF and Na^+ gradients influenced the porter independently with respect to ligand binding and suggested that sodium binding preceded solute binding and translocation.

GROUP TRANSLOCATION

Group translocation processes are unique in that the solute is altered chemically during transport. The best-characterized group translocation systems are the phosphoenolpyruvate-dependent sugar PTSs, which were first discovered by Kundig et al. in *E. coli* (92). PTSs have been the subject of many recent reviews (28, 59, 61, 132, 151). Therefore, in this discussion we concentrate on recent developments concerning PTSs and the distribution of these systems in procaryotes.

Phosphoenolpyruvate:Sugar Phosphotransferase System: Recent Developments

PTS sugar uptake and phosphorylation require the participation of a number of soluble and membrane-bound enzymes (Fig. 2). These proteins catalyze the transfer of the phosphoryl moiety of phosphoenolpyruvate to the sugar substrate. The overall reaction requires Mg^{2+}, and the products formed are sugar phosphate and pyruvate. Two soluble proteins, enzyme I and the low-molecular-weight heat-stable protein HPr, initiate phosphoryl transfer. These are general proteins, and mutations in the genes coding for their syntheses inhibit the uptake of all PTS sugars. Enzyme I and HPr are coded for by the *ptsI* and *ptsH* genes, respectively, both of which map within the *pts* operon. This operon also contains a promoter-like gene, and the levels of these enzymes can be enhanced about threefold by growth on a PTS substrate (29, 163).

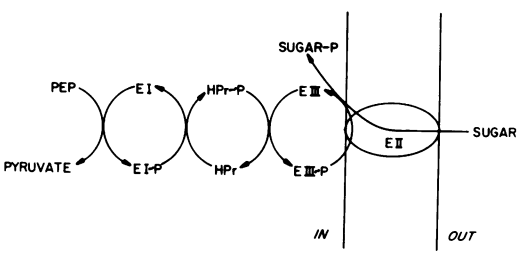

FIG. 2. *Phosphoenolpyruvate:sugar PTS in E. coli and S. typhimurium. Abbreviations: EI, enzyme I; EII, enzyme II; EIII, enzyme III; HPr, small, histidine-containing phosphocarrier protein of the PTS; PEP, phosphoenolpyruvate; ~P, phosphorylated compound.*

Enzyme I has been partially purified from both *E. coli* and *S. typhimurium* cells and has a molecular weight of about 70,000 (132). Enzyme I from *S. aureus* has a molecular weight of approximately 80,000. As indicated in Fig. 2, a phosphorylated form of this enzyme is produced during the transfer of the phosphoryl group from phosphoenolpyruvate to HPr. Studies on the stability of this intermediate indicate that the phosphoryl group is attached to the N-1 or N-3 position of an imidazolé ring of a histidine residue (132).

HPr has been purified to homogeneity from several strains of bacteria (132). *Salmonella* HPr has a molecular weight of 9,500, and it has the same amino acid composition as the *E. coli* protein. Neither protein contains tryptophan, tyrosine, or cysteine, but each has two histidine residues, only one of which is phosphorylated by enzyme I phosphate. The phosphohistidine carries the phosphoryl group at the N-1 position of the imidazole ring (6). HPr was originally so named because it appeared to be heat stable when heated at 100°C. However, it is now known that this treatment causes deamidation, yielding modified proteins of lower specific activity (6).

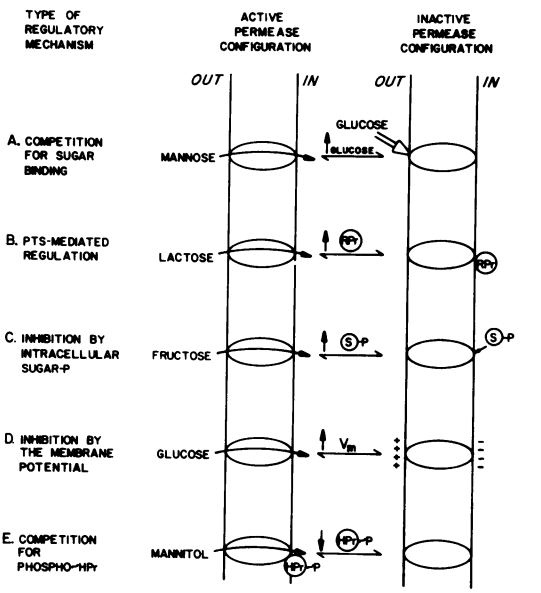

FIG. 3. *Proposed carbohydrate transport regulatory mechanisms in bacteria. Representative examples occur in E. coli. Abbreviations: HPr, histidine-containing protein of PTS; PTS, phosphoenolpyruvate:sugar phosphotransferase system; RPr, regulatory protein (glucose enzyme III [enzyme III^{Glc}] of PTS); sugar-P, sugar phosphate; V_m, V_{max}; S, substrate.*

Recent experiments have shown that enzyme I and HPr catalyze partial reactions in the absence of sugar-specific components of the PTS (M. H. Saier, Jr., M. R. Schmidt, and P. Lin, J. Biol. Chem., in press). Enzyme I was shown to catalyze a rapid phosphoryl exchange reaction in which the phosphoryl moiety of phosphoenolpyruvate was transferred to pyruvate. In addition, enzyme I plus HPr catalyzed the slow hydrolysis of phosphoenolpyruvate to pyruvate and inorganic phosphate. A simple and quantitative assay was developed for measuring these reactions. When ^{14}C-labeled phosphoenolpyruvate or pyruvate was used, the formation of [^{14}C]pyruvate or phosphoenolpyruvate was measured, respectively. The two radioactive products were separated by conversion of pyruvate into the dinitrophenyl hydrazone derivative, which was extracted into ethyl acetate.

By using this assay, it was shown that the enzyme I-catalyzed phosphoryl exchange reaction, as well as the sugar phosphorylation reaction, showed sigmoidal kinetics when the reaction rate was plotted versus enzyme I concentration. This result suggests the possibility that the enzyme undergoes self-association and that the associated form of the enzyme possesses higher activity than the dissociated form.

Enzyme I was activated by Mg^{2+}, Mn^{2+}, and Co^{2+} but not by the other divalent cations tested. The optimum pH in the presence of Mg^{2+} was 7.5. Kinetic binding constants were estimated as follows: phosphoenolpyruvate, 0.25 mM; pyruvate, 2.5 mM; Mg^{2+}, 3 mM; phosphoenolpyruvate bound to phospho-enzyme I, 2.5 mM; pyruvate bound to free enzyme I, 20 mM. Enzyme I catalyzed exchange reactions when α-ketobutyrate or β-hydroxypyruvate replaced pyruvate, but higher homologs of pyruvate were essentially inactive. These results extend the earlier report of Kundig and Roseman (93) and provide a detailed kinetic description of the reaction catalyzed by the first of the energy-coupling enzymes of the PTS.

Further experimentation has provided evidence for a functional association of the soluble proteins of the PTS with the membrane in *E. coli* cells and membrane vesicles. These studies were prompted by the observation that in extracts of *Ancalomicrobium adetum*, both enzyme I and HPr were associated with the particulate fraction (164). In studies with the *E. coli* system, several independent assay procedures have been used to estimate the activities of the enzyme constituents of the PTS in osmotically shocked bacterial membrane vesicles (M. H. Saier, Jr., and D. F. Cox, submitted for publication). The soluble enzymes of the system were determined to be in association with the membrane by several criteria. Phosphoenolpyruvate-dependent sugar phosphorylation was catalyzed by this membrane-bound enzyme complex far more efficiently than by a mixture of the individual enzymes at corresponding concentrations. By contrast, the rates of phosphoryl exchange reactions catalyzed by enzyme I and the enzyme II complexes were essentially the same for the associated and dissociated forms of the complexes. The functional association of the PTS enzymes with the membrane could be destroyed by detergent treatment, sonication, or passage of the vesicle preparation through a French pressure cell.

The PTS enzyme complex was stabilized by Mg^{2+} and phosphoenolpyruvate and destabilized by divalent cation chelating agents. The disruptive effects of the detergents were shown to be essentially irreversible and were not due to inhibition of the enzyme activities during assay. These results lead to the possibility that in intact bacterial cells the soluble enzymes of the PTS exist, at least in part, as peripheral membrane constituents in association with integral membrane enzyme II complexes.

The sugar-specific PTS proteins are enzymes II and III and are associated with the cytoplasmic membrane. Enzyme III is a peripheral membrane protein, whereas enzyme II complexes are integral components of the membrane. Kundig (91) reported the purification of enzyme III^{glc} to apparent homogeneity. However, the homogeneity of this protein preparation is questionable since it retained hexose-6-phosphate phosphatase activity. Enzyme III^{glc} has an apparent molecular weight of 20,000, as determined by gel filtration (91). An acyl group in this protein appears to be phosphorylated, based on chemical identification of the phosphorylated residue after hydrolysis. This enzyme may be coded for by the *crrA* gene, which lies very close to the *pts* operon in both *E. coli* and *S. typhimurium* (28, 151).

Extensive experimental evidence favors the notion that the integral membrane enzyme II complexes serve as the sugar recognition components of the system. Until recently, however, none of these enzymes had been obtained in pure form, and we had little information concerning the mechanism by which they catalyze transmembrane sugar translocation. The discovery of the sugar phosphate-sugar transphosphorylation reactions catalyzed by these enzymes has led to a more detailed concept of the mechanism by which they act upon their substrates (153). This is due to the fact that the transphosphorylation reactions are thought to occur in a

vectorial sense, as follows:

$$^{14}\text{C-sugar}_{out} + \text{sugar phosphate}_{in} \rightleftharpoons {}^{14}\text{C-sugar phosphate}_{in} + \text{sugar}_{out}$$

A particular enzyme II exhibits strict specificity toward the sugar, as well as the sugar phosphate substrate.

The transphosphorylation reactions catalyzed by the glucose enzyme II and the mannose enzyme II have been subjected to detailed kinetic analyses (140). The reaction mechanism demonstrated for both enzymes was found to be Bi-Bi sequential, indicating that each enzyme II possesses nonoverlapping binding sites for sugar and sugar phosphate. Although there appeared to be no preferred order of substrate binding, association of the two substrates with the enzyme occurred in a positively cooperative fashion; that is, binding of the sugar-phosphate substrate to the enzyme II complex apparently enhanced the affinity of the enzyme for the sugar substrate and vice versa. A mutant with a defective glucose enzyme II was isolated, which transported methyl-α-glucoside and glucose with reduced maximal velocities and higher K_m values. In vitro kinetic studies of the transphosphorylation reaction catalyzed by the mutant enzyme showed decreased maximal velocities and increased K_m values for both the sugar and sugar phosphate substrates. Therefore, it was concluded that a single enzyme II complex catalyzed both transport and transphosphorylation of its sugar substrates.

Recently, this conclusion has been substantiated with homogeneous mannitol enzyme II (70). Purified detergent-solubilized mannitol enzyme II exhibited a subunit molecular weight of about 60,000; it catalyzed both phosphoenolpyruvate-dependent phosphorylation of [^{14}C]mannitol in the presence of the soluble enzymes of the PTS (enzyme I and HPr) and mannitol 1-phosphate-[^{14}C]mannitol transphosphorylation in the absence of the soluble enzymes. That mannitol enzyme II is the only membrane protein encoded by the mannitol operon in *E. coli* was also demonstrated. In these experiments, a hybrid ColE1 plasmid carrying the mannitol operon was transferred to a minicell-producing strain of *E. coli*. The minicells, which were free of chromosomal deoxyribonucleic acid, synthesized only two proteins in response to the inducer (mannitol) and cyclic adenosine 5'-monophosphate (cyclic AMP). One such protein was membrane bound, had a molecular weight of 60,000, and was presumed to be mannitol enzyme II. The other protein was soluble, had a protomer molecular weight of 40,000, and was presumed to be mannitol phosphate dehydrogenase. These results suggest the probability that an enzyme II complex consisting of a single polypeptide chain (possibly in an oligomeric complex) in association with membrane phospholipids catalyzes phosphoenolpyruvate-dependent sugar uptake and phosphorylation, as well as sugar phosphate-sugar exchange group translocation (153).

Recent unpublished data from the laboratory of Hengstenberg have indicated that, in contrast to results reported previously (89), the membrane-bound enzyme II of the lactose PTS in *S. aureus* has, like *E. coli* mannitol enzyme II, a fairly high molecular weight. The inactive enzyme was purified to near homogeneity after treatment with a high concentration of sodium dodecyl sulfate. The protein had a protomer molecular weight of about 55,000 (W. Hengstenberg, personal communication). Antibodies prepared against the purified polypeptide chain completely inhibited the phosphorylation of lactose when crude membranes served as the source of the lactose enzyme II (enzyme II^{lac}). Moreover, synthesis of the protein was induced by growth in the presence of β-galactosides, as was expression of the lactose operon. These experiments showed that the enzyme II^{lac} from *S. aureus* is similar in size to *E. coli* mannitol enzyme II. Since enzyme II^{lac} also catalyzes both sugar phosphate-sugar transphosphorylation and phosphoenolpyruvate-dependent sugar phosphorylation, the mechanisms of transport catalyzed by the two enzymes are probably similar.

Distribution of Phosphotransferase Systems in Bacteria

PTSs are widely distributed in procaryotes; Table 3 shows the bacterial genera in which PTSs have been found. Also given are the Bergey group numbers for the various genera and the known PTS substrates. Table 4 lists the bacterial genera in which PTS activity has been sought but not found. This distribution follows two general patterns, depending on the metabolic pathway through which hexoses and hexitols are broken down. These are as follows.

(i) Strict aerobes, such as *Azotobacter*, *Micrococcus*, *Mycobacterium*, and *Nocardia*, do not possess PTSs. Obligately aerobic organisms usually metabolize hexoses and hexitols via the Entner-Doudoroff pathway (N. Pelliccione, B. Jaffin, M. E. Sobel, and T. A. Krulwich, Eur. J. Biochem., in press), in which the primary energy sources generated are ATP and a proton-electrochemical gradient. Therefore, it is more advantageous for aerobes to use these forms of energy to drive transport systems directly rather than

go through a potentially wasteful transformation process in order to generate phosphoenolpyruvate. There are several exceptions to this aerobic pattern of PTS distribution. Several *Bacillus* species and *Arthrobacter pyridinolis* have general PTSs capable of phosphorylating a variety of hexoses and hexitols (25, 33, 99, 184; Pelliccione et al., in press). These organisms metabolize sugars via the Embden-Meyerhof pathway (186), the products of which render transport via the PTS beneficial (see below).

(ii) Most facultative anaerobes and some obligate anaerobes possess a PTS if sugar is metabolized via anaerobic glycolysis (i.e., Embden-Meyerhof pathway). Hexoses metabolized through this pathway yield 2 mol of phosphoenolpyruvate per mol of hexose utilized. Consequently, uptake of hexoses by the PTS and metabolism through anaerobic glycolysis provides a self-fueling system for transport and at the same time supplies energy in the form of phosphoenolpyruvate, which can be easily transformed to other high-energy compounds. Among the genera of facultative anaerobes known to possess the PTS are *Escherichia, Salmonella, Staphylococcus, Photobacterium,* and *Beneckea. Clostridium* and *Fusobacterium* are examples of obligate anaerobes that possess the PTS, and *Megasphaera,* a microaerophilic rumen organism, has recently been found to have a phosphoenolpyruvate:sugar PTS specific for glucose and fructose (S. S. Dills, C. A. Lee, and M. H. Saier, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, K95, p. 142). As with the aerobic pattern described above, there are several exceptions to this facultatively anaerobic pattern. Heterofermentative species of *Lactobacillus* and *Leuconostoc* which metabolize hexoses via the phosphoketolase pathway have been found to lack PTS activity (147). Similarly, *Zymomonas mobilis,* which utilizes the Entner-Doudoroff pathway for hexose catabolism, does not possess the PTS (59, 147).

Several seemingly divergent organisms have the common characteristic of possessing a single fructose-specific PTS. Representative genera include *Rhodopseudomonas, Pseudomonas,* and *Fusobacterium* (Table 3). Fructose is phosphorylated in the C-1 position, whereas other hexoses are phosphorylated in the C-6 position. This characteristic, along with its unique presence in many divergent organisms, suggests that the fructose-specific PTS had an early origin during the evolution of transport systems.

The pathways of fructose metabolism have been investigated in *Rhodopseudomonas cap-*

TABLE 3. *Distribution of PTS in procaryotes*

Genus	Bergey group	Gram stain reaction	Substrate(s)	Reference(s)
Rhodopseudomonas	1	−	Fructose	158
Rhodospirillum	1	−	Fructose	158
Thiocapsa	1	−	Fructose	26, 27
Thiocystis	1	−	Fructose	26
Ancalomicrobium	4	−	General	164
Spirochaeta	5	−	Mannitol	160
Pseudomonas	7	−	Fructose	10, 170
Alcaligenes	7	−	Fructose	169
Beneckea	8	−	General	52
Escherichia	8	−	General	132
Klebsiella	8	−	General	190
Photobacterium	8	−	General	52
Salmonella	8	−	General	132
Serratia	8	−	General	P. Talalay and M. H. Saier[a]
Fusobacterium	9	−	Fructose	138
Megasphaera	11	−	Glucose, fructose	Dills et al.[b]
Staphylococcus	14	+	General	39, 50
Streptococcus	14	+	General	112, 115, 171, 191
Bacillus	15	+	General	33, 56, 146
Clostridium	15	+	Fructose, mannitol	129, 200
Brochotrix	16	+	General	B. Mitchener and F. H. Grau[a]
Lactobacillus	16	+	General	105, 147
Arthrobacter	17	+	Fructose, rhamnose	99, 184; Pelliccione et al.[c]
Mycoplasma	19		General	22

[a] Unpublished data.
[b] Dills et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, K95, p. 142.
[c] Pelliccione et al., in press.

TABLE 4. *Bacterial genera thought to lack the PTS*

Genus	Group	Gram stain reaction	Reference(s)
Rhodomicrobium	1	−	158
Caulobacter	4	−	164
Hyphomicrobium	4	?	164
Prosthecomicrobium	4	?	164
Azotobacter	7	−	146
Zymomonas	8	−	147; Romano and Trifone[a]
Bacteriodes	9	−	69
Thiobacillus	12	−	151
Leuconostoc (heterofermentative species)	14	+	147; Romano and Trifone[a]
Micrococcus	14	+	146
Lactobacillus (heterofermentative species)	16	+	147; Romano and Trifone[a]
Mycobacterium	17	+	146
Nocardia	17	+	151

[a] A. H. Romano and J. Trifone, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, K103, p. 153.

sulata (25) and several species of *Pseudomonas* (9, 170, 197). In all cases, fructose enters the cells via the PTS and then is metabolized through either the Entner-Doudoroff or the Embden-Meyerhof pathway. In *R. capsulata* (25) fructose is catabolized exclusively by anaerobic glycolysis. In *Pseudomonas* both pathways are utilized; however, most of the sugar appears to be metabolized via the Entner-Doudoroff pathway (170, 197). This allows the speculation that fructose metabolized via the Entner-Doudoroff pathway is used to supply energy for other cellular processes.

London and Chace (105) have reported the existence of a novel PTS for pentitols in the homofermentative organism *Lactobacillus casei*. These bacteria transport xylitol, arabitol, and ribitol via a phosphoenolpyruvate-dependent phosphotransferase route. Previously, pentitol-specific PTSs had not been described.

REGULATION OF CARBOHYDRATE UPTAKE IN BACTERIA

In 1967 McGinnis and Paigen measured the rates of utilization of ^{14}C-labeled carbohydrates in the presence and absence of a second nonradioactive carbohydrate (113). They demonstrated that glucose caused an immediate and reversible inhibition of ^{14}C-labeled sugar utilization. In a subsequent publication these same investigators proposed that glucose exerted its inhibitory effects on carbohydrate uptake systems (114). However, the mechanisms of inhibition were not considered.

As a result of studies conducted during the past decade, five regulatory mechanisms which appear to account for the control of carbohydrate uptake via various permease systems can be proposed. These include (i) inhibition due to competition between two sugars for sugar binding to the substrate recognition site of a permease protein, (ii) PTS-mediated regulation involving the allosteric inhibition of permease activity by a protein of the PTS, (iii) inhibition of carbohydrate uptake by intracellular sugar phosphates, (iv) chemiosmotic control of transport activity due to responsiveness of the permease system to the membrane potential and/or the intracellular pH, and (v) inhibition of the uptake of one PTS sugar substrate by another sugar substrate of this system due to competition of the corresponding enzyme II complexes for the common phosphoryl protein, phospho-HPr. These mechanisms are illustrated schematically in Fig. 3. The subject of carbohydrate transport regulation was reviewed recently (159), and the present discussion is meant to supplement and update that review.

Competition for Sugar Binding to Permease Proteins

One of the carbohydrates studied by McGinnis and Paigen (113) was [^{14}C]mannose. Its utilization was rapidly inhibited upon addition of glucose to the culture medium. We now know that glucose is transported across *E. coli* cytoplasmic membranes by two of the enzyme II complexes of the PTS. One of these enzymes (enzyme II^{glc}) exhibits fairly high specificity toward glucose and its glycosides, whereas the other (mannose enzyme II [enzyme II^{man}]) is relatively nonspecific with respect to the substituent on carbon 2 (132, 140). The latter enzyme transports glucose, mannose, 2-deoxyglucose, *N*-acetylglucosamine, glucosamine, and fructose with decreasing affinity in that order (140). When enzyme II^{glc} was eliminated by a genetic lesion in the structural gene for this protein, glucose still effectively inhibited uptake of the other substrates of enzyme II^{man} (A. W. Rephaeli and M. H. Saier, Jr., unpublished data). Inhibition occurred by a competitive mechanism in which glucose inhibited the binding of mannose to the sugar binding site of enzyme II^{man}. The approximate K_m values (apparent affinities) of the enzyme for glucose and mannose, as estimated from kinetic analyses of ^{14}C-labeled sugar uptake, were 13 and 20 μM, respectively (140). Thus, competition for sugar binding to enzyme II^{man} was largely responsible for inhibition of mannose uptake by glucose in the mutant bacterial strain which lacked enzyme II^{glc} and was a major reason for inhibition of mannose uptake in the wild-type strain.

Phosphotransferase System-Mediated Regulation

The evidence for direct catalytic participation of the proteins of the PTS in the regulation of the uptake of certain carbohydrates which are not substrates of the PTS (non-PTS sugars) has been reviewed previously (151, 155, 159). Recently, the regulation of lactose uptake in membrane vesicles of *E. coli* was studied (S. S. Dills and M. H. Saier, Jr., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K7, p. 146). The results can be summarized as follows.

PTS substrates inhibited lactose uptake by membrane vesicles prepared from *E. coli* strain ML 308-225 cells. This inhibition was relieved by osmotically shocking phosphoenolpyruvate into the vesicles. By using the same conditions, PTS substrate uptake was stimulated by intravesicular phosphoenolpyruvate. In addition, methyl-α-glucoside uptake in the presence of phosphoenolpyruvate was stimulated further when either HPr or a partially purified preparation of enzyme III^{glc} was osmotically shocked into the vesicles. By contrast, intravesicular enzyme III^{glc} inhibited lactose uptake, whereas HPr was not inhibitory. The decrease in lactose accumulation caused by the enzyme III^{glc} preparation was partially overcome by the addition of phosphoenolpyruvate. In the presence of phosphoenolpyruvate, HPr stimulated vesicular lactose accumulation. These results support the conclusion that the PTS proteins function catalytically in the regulation of carbohydrate uptake. They also provide the first direct biochemical evidence supporting the notion that an allosteric effector, the proposed regulatory protein RPr, is in fact enzyme III^{glc}, as suggested by earlier genetic experiments (155, 159).

Recent studies have revealed that the PTS-mediated regulatory system is itself subject to regulation. This secondary control has been termed "desensitization" (D. K. Keeler, B. U. Feucht, and M. H. Saier, Jr., Fed. Proc. **36**:685, 1977). When *E. coli* or *S. typhimurium* cells were grown in the presence of cyclic AMP and the exogenous inducers of the transport systems specific for glycerol, maltose, melibiose, or lactose, the corresponding transport system became resistant to inhibition by PTS sugar substrates. Inducer-promoted desensitization was specific for a particular target permease. The cyclic AMP response was specific to this nucleotide and was potentiated by the genetic loss of cyclic AMP phosphodiesterase. Cyclic AMP-promoted desensitization was general for all transport systems subject to PTS-mediated regulation.

The extent of desensitization increased slowly during cell growth in the presence of inducer and/or cyclic AMP and was blocked by chloramphenicol, suggesting that protein synthesis was required. Desensitization appeared to uncouple the target permease systems from regulation without altering the activity of enzyme I, HPr, or one of the enzyme II complexes of the PTS. Evidence regarding the desensitization mechanism came from studies of lactose permease desensitization as a function of the degree of induction of the *lac* operon in *E. coli*. When expression of the operon was only partially induced, the sensitivity of lactose uptake to PTS-mediated regulation was strong. Also, under partial induction conditions the activities of β-galactosidase and the lactose permease were induced in parallel. By contrast, when the degree of induction was more extensive, the ratio of lactose permease to β-galactosidase activity decreased (Dills and Saier, unpublished data). Transacetylase activity paralleled β-galactosidase activity. Since all three proteins are coded for by a single polycistronic messenger ribonucleic acid, it was assumed that they were synthesized in parallel. If all of the induced permease proteins were inserted into the cytoplasmic membrane in a potentially functional fashion but another essential transport component (such as cellular energy) was rate limiting for lactose uptake, then the ratio of apparent lactose permease activity to β-galactosidase activity would be expected to decline. Moreover, if the binding of a limited number of RPr molecules to the lactose permease proteins was determined by the total number of carbohydrate permease proteins present (both active and inactive), then the percentage of active lactose permease molecules which were inhibited by RPr would decrease with increasing degrees of induction. Desensitization would be enhanced further if RPr binding to a permease protein converted it to an inactive form, thereby shunting the rate-limiting transport component to permeases to which RPr was not bound. These mechanistic considerations appear to be consistent with the preliminary evidence cited above. Further research will be required to establish or refute these hypotheses.

The discovery of bacterial desensitization has provided explanations for several previously anomalous observations. Pastan and Perlman (128) and others (16, 161; W. Epstein, S. Jewett, and R. H. Winter, Fed. Proc. **29**:601, 1970) reported that cyclic AMP allowed maximal rates of synthesis of enzymes sensitive to catabolite repression in the presence of an inducer and glucose. It was assumed that exogenous cyclic AMP exerted its effect in overcoming glucose

repression by correcting a cyclic AMP deficiency (128). However, later studies revealed that PTS-mediated repression of non-PTS carbohydrate catabolic enzyme synthesis in *S. typhimurium* and some *E. coli* strains was primarily due to inhibition of inducer uptake rather than to a cyclic AMP deficiency (154, 166). The explanation of the cyclic AMP effect appears to be as follows. Cyclic AMP induces desensitization of the permease to regulation, and desensitization abolishes inducer exclusion. Cytoplasmic inducer concentrations can then approach the optimal value for enzyme induction. Exogenously added cyclic AMP therefore abolishes enzyme synthesis repression both by correcting a cyclic AMP deficiency and by abolishing inducer exclusion.

The physiological significance of permease desensitization appears to be clear. Intracellular cyclic AMP levels normally serve a bacterium as an indicator of carbon and energy sufficiency. When carbon sources are plentiful, internal cyclic AMP levels are depressed. Conversely, carbon starvation results in an enhancement of the cellular concentration of this cyclic nucleotide (109, 127). If several carbohydrates are present in the medium at concentrations which are substantially below their transport K_m values, acquisition of enough carbon for rapid growth requires that the permeases for more than one sugar be induced to high levels and function at maximal rates. If both a PTS and a non-PTS exogenous carbohydrate are present, simultaneous and rapid uptake of the two carbohydrates requires abolition of the PTS-mediated inhibitory mechanism. Because the total concentration of exogenous carbohydrates is low, cyclic AMP synthetic rates are high, and rates of efflux of the cyclic nucleotide are low (109, 127, 157). Consequently, cyclic AMP accumulates in the cytoplasm, enhancing the degree of permease induction and causing desensitization. It is likely that desensitization will prove to be an important regulatory process in natural environments where bacterial cells are exposed alternately to feast and famine conditions (85).

Inhibition of Sugar Uptake by Intracellular Sugar Phosphates

Several investigators, particularly Kornberg et al., have presented evidence for a mechanism of carbohydrate transport regulation which involves intracellular carbohydrate phosphates as the transport inhibitors (5, 86, 159, 162). A wide variety of carbohydrate permeases appear to show decreased activity when the concentrations of intracellular metabolites such as sugar phosphates are elevated. Among these permeases in *E. coli* are those specific for lactose (which enters the cell by proton symport), for fructose (a PTS substrate), and for glycerol (which crosses the membrane by facilitated diffusion). Thus, transport systems which utilize different energy-coupling mechanisms are subject to this regulatory constraint. In addition, inhibition of permease activity by internal sugar phosphates has been demonstrated in gram-positive as well as gram-negative bacteria (162). The evidence for this type of regulation is not considered here, since there is no information in addition to that discussed by Kornberg (86) and Saier and Moczydlowski (159).

The mechanism by which intracellular carbohydrate phosphates inhibit sugar uptake is not known. The sugar phosphates could interact directly with an allosteric regulatory site on the cytoplasmic surface of the cell membrane, thereby inhibiting permease activity. Alternatively, the inhibitory effects could be indirect (for example, by interfering with energy-yielding or energy-coupling processes). Other nonspecific interactions which decrease permease activity can also be envisaged. Further experimentation will be required to distinguish among these possibilities.

Control of Permease Activities by the Energized Membrane State

As a result of recent experimentation in several laboratories, it is clear that bacterial permeases may be responsive to the proton electrochemical gradient (PMF) either in a positive or a negative sense. Regulation of the activity of the glucose enzyme II represents an example of a transport system which appears to be subject to negative control by the PMF (54, 62, 159).

Recently, Reider et al. provided confirmation for this suggestion (139). Methyl-α-glucoside uptake was studied in both intact *E. coli* cells and membrane vesicles. The imposition of a proton gradient across the membranes inhibited sugar uptake, and this inhibition was overcome by adding proton-conducting uncouplers. The inhibition of sugar uptake due to energization by respiration of D-lactate in vesicles was reversed by oxamate (an inhibitor of D-lactate dehydrogenase) and cyanide (an inhibitor of electron flow), as well as by the uncouplers. Vesicles derived from a mutant lacking cytochrome were not energized by D-lactate oxidation, and methyl-α-glucoside uptake was not inhibited. Uptake of other substrates of the glucose enzyme II and uptake of substrates of the mannose enzyme II were inhibited similarly by the energized membrane state. These results explain the enhancement of methyl-α-glucoside uptake upon addition of colicins Ia, K, and E to intact *E. coli* cells and upon infection of the cells with

phage T1. All of these agents have been shown to dissipate the energized membrane state.

Saier presented preliminary evidence that citrate uptake in *S. typhimurium* is subject to positive regulation by some form of chemiosmotic energy (152). This proposal has been strengthened recently by evidence which suggests that two other *E. coli* permeases are subject to regulation by chemiosmotic energy. The major "pore" protein in the *E. coli* outer membrane responds negatively, whereas the proton-translocating ATPase (F_0-F_1 complex), which is present in the inner membrane, responds in a positive fashion to the membrane potential. In addition, amino acid transport systems in *Halobacterium halobium* exhibit behavior which can be explained partially in terms of complex regulatory interactions involving the membrane potential.

The matrix protein of the *E. coli* outer membrane forms nonspecific aqueous channels, which allow passage of small hydrophilic molecules through the phospholipid bilayer (172; J. E. Leonard, C. Lee, A. Apperson, S. S. Dills, and M. H. Saier, Jr., *in* B. K. Ghosh, ed., *Membrane Structures in Bacterial Cells*, in press). The dependence of solute transport via these channels on transmembrane voltage was demonstrated after incorporation of the pure protein into planar lipid bilayers. It was suggested (172) that the channels formed by this protein could assume either an open or a closed configuration or state and that the state of the protein was influenced by the membrane potential. Since an increase in the transmembrane voltage shifted the presumed two-state equilibrium distribution toward the closed configuration, this transport system exhibited a negative response to voltage. This response is qualitatively similar to that observed for the excitability-inducing material, a bacterial protein preparation of unknown function which conceivably plays a role in forming aqueous pores through the outer membranes of gram-negative bacterial cell envelopes (165). The response is also qualitatively similar to that observed for the transport of monovalent cations across the nerve cell membranes of higher animals (165).

A bacterial permease system which responds to the proton electrochemical potential (the PMF) in a positive sense is the proton-translocating ATPase (F_0-F_1 complex) in the cytoplasmic membrane of *Streptococcus lactis* (110; Leonard et al., in press). The ATPase complex apparently transports protons only after the PMF has reached a certain threshold value (110). Thus, the transport channel displays a positively gated response. Both in this case and in that of the outer membrane matrix protein, it is thought that the transmembrane channel is an oligomeric structure which is formed by the aggregation of identical protein protomers (172; Leonard et al., in press). Thus, the membrane potential could influence pore size by altering either the conformation of the subunit or the propensity of the monomer to form functional aggregates.

Recently, Lanyi et al. reported the apparently cooperative dependencies of amino acid transport in *H. halobium* on the chemical and electrical components of the Na^+ gradient (98, 108). In this organism, amino acid uptake was energized by Na^+ cotransport (108). The initial rates of transport of aspartate and serine were fourth-power functions of the electrical component of the Na^+ electrochemical potential, although uptakes were first-power (for aspartate) and second-power (for serine) functions of the Na^+ chemical gradient. V_{max} values, but not K_m values, showed dependencies on the membrane potential (98), as was observed for the citrate permease in *Salmonella* (152). It appears that these transport systems provide additional examples of permeases for which chemiosmotic energy influences the functional states and hence the maximal activities of the permease protein.

Competition for Phospho-HPr: Regulation of Sugar Uptake via the Phosphotransferase System

Recent experiments have shown that the uptake of one sugar substrate of a bacterial PTS can inhibit the uptake of a second substrate of the system by a mechanism that has been attributed to competition of the different enzyme II complexes for the common phosphoryl donor, phospho-HPr (M. H. Saier, Jr., and H. L. Kornberg, unpublished data). This conclusion was first suggested by experiments with *S. typhimurium* strains, which showed that inhibition of fructose uptake by methyl-α-glucoside was enhanced by a reduction in cellular enzyme I activity or by energy depletion (Fig. 4). When the enzyme I-deficient strain *ptsI17* was used (154), strong and immediate inhibition of fructose uptake by methyl-α-glucoside was observed, even though this mutant accumulated intracellular methyl-α-glucoside phosphate at a slow rate (Fig. 4A and D). By contrast, in the presence of an exogenous energy source, the parental strain accumulated methyl-α-glucoside phosphate rapidly, but no inhibition of fructose uptake was observed (Fig. 4C and F). Only when this strain was depleted of energy did methyl-α-glucoside exert an inhibitory effect (Fig. 4B and E). It is worth noting that there was an inverse relation-

ship between methyl-α-glucoside phosphate accumulation (Fig. 4A through C) and sensitivity of fructose uptake to inhibition by the glycoside (Fig. 4D through F) when either the cellular energy or the enzyme I content was varied. Since enzyme I catalyzed the phosphorylation of HPr in the presence of phosphoenolpyruvate (the energy source for sugar uptake), these results are consistent with a mechanism in which two enzyme II complexes compete for phospho-HPr binding.

A more extensive study, involving six different substrates of the PTS both as uptake substrates and as inhibitors, revealed that any natural sugar substrate of the PTS could inhibit the uptake of any other substrate in strain *ptsI17* cells, provided that the enzyme II specific for the inhibitory sugar was present in sufficient amounts. The *crrA1* mutation, which abolished regulation of non-PTS permeases by the PTS in the *ptsI17* genetic background (154), had virtually no effect on the regulation of fructose uptake by other substrates of the PTS.

It has been reported that in *E. coli* a mutation (designated *crr*) which maps near the *pts* operon and a mutation (designated *cif*) which maps in or near the fructose regulon partially abolish the inhibitory effect of methyl-α-glucoside on fructose uptake (5, 88). In preliminary studies, an isogenic series of *E. coli* mutants were examined with the following results. (i) Although the *crr* mutation released fructose uptake from inhibition by glucose and methyl-α-glucoside (transported by glucose enzyme II) to a greater extent than did the *cif* mutation, inhibition by *N*-acetylglucosamine (transported by *N*-acetylglucosamine enzyme II) was relieved by the *cif* mutation to a much greater extent than by the *crr* mutation. (ii) The *cif* mutation but not the *crr* mutation relieved inhibition of fructose uptake by glucose 6-phosphate and 2-deoxyglucose 6-phosphate when these exogenously added sugar phosphates were transported by the hexose phosphate permease (34). (iii) The *crr* mutation in this genetic background not only relieved inhibition of fructose uptake by methyl-α-glucoside, it also decreased the total methyl-α-glucoside uptake and significantly enhanced inhibition of methyl-α-glucoside uptake by fructose. The last effect was not observed with the *cif* mutants (P. Watts, M. H. Saier, Jr., and H. L. Kornberg, unpublished data). These observations suggest that *crr* mutations in *E. coli* may relieve the inhibition of fructose uptake by methyl-α-glucoside both by reducing the affinity of the glucose enzyme II complex for phospho-HPr and by reducing the extent of methyl-α-glucoside phosphate accumulation in the presence of fructose. This effect is apparently specific to substrates of glucose enzyme II, although *crrA* mutations in both *S. typhimurium* and *E. coli* have been shown to prevent PTS-mediated regulation of non-PTS permeases by all sugar substrates of the PTS (154, 159, 161). Taken together, these results suggest that competition for phospho-HPr provides a mechanism for the fine control of PTS sugar utilization in both *S. typhimurium* and *E. coli*.

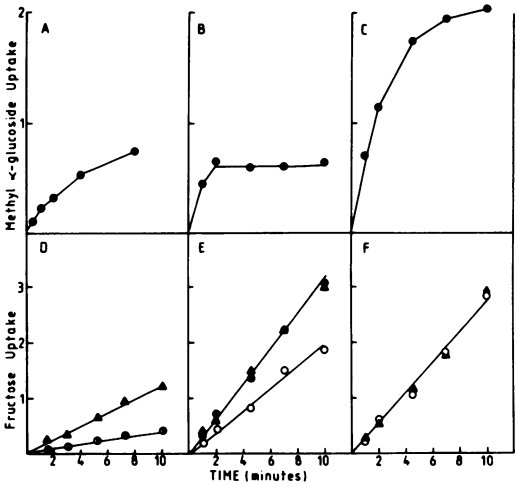

FIG. 4. *Uptake of ^{14}C-labeled methyl-α-glucoside and [^{14}C]fructose by S. typhimurium strains. Strain ptsI17 cells (A and D) were grown in nutrient medium supplemented with 0.5% fructose. Strain LT-2 cells (B, C, E, and F) were grown in the same medium, but glucose (0.5%) was added to the cell suspension 2 h before harvesting. Preparation of cells and uptake measurements were conducted as described previously. (A through C) Uptake of ^{14}C-labeled methyl-α-glucoside (40 μM). (D through F) Uptake of [^{14}C]-fructose (10 μM). Uptake measurements in (A), (B), (D), and (E) were conducted in half-strength medium 63 without the addition of an exogenous energy source. Nutrient broth (3 g/liter) was added to the uptake medium in the experiments shown in (C) and (F). In (D) through (F) [^{14}C]fructose uptake was measured in the absence of methyl-α-glucoside (▲), or this glucose analog was added together with (●) or 10 min before (○) the radioactive substrate.*

Inducer Expulsion

In addition to the five regulatory mechanisms discussed above, a novel type of transport regulation has been demonstrated recently in *Streptococcus pyogenes* (J. Reizer and C. Panos, personal communication). This organism accumulates β-galactosides as the phosphate esters due to a phosphotransferase-catalyzed uptake process. The addition of glucose to a cell suspension

of *S. pyogenes* which had accumulated TMG phosphate induced rapid expulsion of free TMG from the intracellular sugar phosphate pool. Normal substrates of the lactose phosphotransferase did not promote rapid efflux, and nonmetabolizable glucose analogs were essentially without effect. Similar observations have been made in *S. lactis*, in which two TMG transport systems have been demonstrated; one of these systems is a PTS-dependent lactose permease, and the other is a galactose-specific active transport system. A role for both transport systems in the TMG phosphate exclusion process has been suggested (J. Thompson, personal communication). Although a mechanistic explanation for these interesting findings is not currently available, a possible physiological function of the phenomenon has been advanced (Reizer and Panos, personal communication). Because internal galactoside phosphate apparently functions as the true inducer of the lactose regulon in *S. aureus* (59, 132), displacement of intracellular TMG phosphate can be regarded as inducer expulsion. Inducer expulsion is distinct from inducer exclusion, which results from inhibition of sugar uptake. The former process may have the physiological advantage of more rapidly diminishing inducer concentrations, thereby arresting enzyme synthesis more effectively. Further studies will be required to elucidate the mechanism of inducer expulsion in streptococcal species.

EXOGENOUS INDUCTION OF BACTERIAL PERMEASE SYNTHESIS

The classic induction mechanism involving an intracellular repressor protein, the conformation of which is modified by a small inducer, is typified by the well-characterized *lac* system (11). In this system, a β-galactoside is transported into an uninduced cell via one of the few functional permease molecules present in the cell membrane. The intracellular β-galactoside then binds to the repressor of the lactose operon, thereby altering its conformation and decreasing its affinity for the *lac* operator. Transcription of the *lac* genes and subsequent translation of the *lac* messenger ribonucleic acids into proteins can then occur. Similar intracellular induction mechanisms involving positive control proteins have been established for other carbohydrate metabolic systems, including those involved in the utilization of maltose and arabinose (41).

Proteins Induced by Exogenous Inducers

There is another class of proteins, whose synthesis is induced by fundamentally different mechanisms. These proteins are induced only when the inducer is present on the external surface of a cell. There is evidence which indicates that several different protein systems are induced exogenously (Table 5).

The most recent additions to the list of systems induced exogenously are the proteins of the PTS; these include the *pts* operon, which codes for HPr and enzyme I, and the genes *glu* (*ptsG*) and *manA* (*ptsM*), which in *S. typhimurium* and *E. coli* code for enzyme II^{glc} and enzyme II^{man}, respectively. The *pts* operon and the genes coding for these enzymes II were shown to be induced maximally by glucose. However, when uninduced cells were grown on one of the disaccharides maltose or melibiose, which release free glucose inside the cell, no induction occurred. This result indicates that intracellular glucose is not responsible for induction of these phosphotransferase proteins (141).

The phosphoglycerate permease of *S. typhimurium* transports and is induced by 3-phosphoglycerate, 2-phosphoglycerate, and phosphoenolpyruvate (167). Induction of this system occurs when one of these compounds is present in the growth medium at a concentration of 100 μM (167) despite the fact that log-phase cell cultures contain these three inducers at concentrations of about 1 mM (145). Since the transport system remains uninduced until one of the substances is added externally, a mechanism involving induction from without is implied.

The tricarboxylic acid permease system of *S. typhimurium* transports the following Krebs cycle tricarboxylates: citrate, isocitrate, tricarballylate, and *cis*- and *trans*-aconitate (76). Induction of this system appears to be restricted to citrate, isocitrate, and tricarballylate (76, 152). As in the case of the phosphoglycerate transport system, the inducers of the system are normal metabolic intermediates which are present within growing cells. However, even in aconitase-negative mutants (73), in which citrate is accumulated, induction only occurs upon addition of citrate to the external medium. A requirement for extracellular induction was also observed in isocitrate dehydrogenase mutants of *S. typhimurium* (W. W. Kay, personal communication). Under similar conditions, however, an aconitase mutant of *B. subtilis* showed gratuitous induction (203).

The transport system for which the most extensive evidence for extracellular induction has been obtained is the hexose phosphate permease of *E. coli* and *S. typhimurium*. This system (genetic designation, *uhp*) was first identified in 1966 by Pogell and co-workers (131) and Winkler (213). It is fairly nonspecific with regard to its substrates, transporting several hexose phos-

TABLE 5. *Synthesis of transport proteins in response to exogenous inducers*

Gene designation(s)	Organism	Gene product(s)
uhp	*E. coli*	Hexose phosphate transport system
pgt	*S. typhimurium*	Phosphoglycerate transport system
pts	*S. typhimurium*	Enzyme I and HPr of the PTS
glu, manA	*S. typhimurium*	Enzyme II^{glc} and enzyme II^{man} of the PTS
tct	*S. typhimurium*	Tricarboxylate transport system
pho	*E. coli*	Alkaline phosphatase

phates (131, 213) as well as sedoheptulose phosphate and a pentose phosphate (40). However, of these substrates only glucose 6-phosphate and its non-metabolizable analog 2-deoxyglucose 6-phosphate serve as inducers.

Several independent lines of evidence support the conclusion that this system is responsive only to extracellular inducer (34, 36, 68, 214). As in the cases of the other extracellularly induced systems mentioned above, the steady-state concentration of inducer within uninduced log-phase cells is greater than the external concentration needed for induction. For example, the internal glucose 6-phosphate concentration of uninduced cells growing on glucose or glycerol is 0.5 to 2.8 mM (68), but the concentration of glucose 6-phosphate in the growth medium required for induction of the *uhp* regulon is 0.1 mM (34). The use of a double mutant defective for hexose phosphate isomerase and glucose-6-phosphate dehydrogenase reinforced this observation (214). When this mutant was grown in the presence of glucose, the internal glucose 6-phosphate concentration reached 60 mM, yet the *uhp* genes were not expressed. The addition of 0.5 mM glucose 6-phosphate to the medium induced the hexose phosphate permease, thereby releasing the growth stasis caused by the accumulated sugar phosphate when its efflux from the cell ensued (34).

A comparison of the apparent affinity constants for induction by and transport of glucose 6-phosphate further substantiated the conclusion that an extracellular induction mechanism was operative. The apparent K_m for glucose 6-phosphate uptake was reported to be 500 μM. Under the same conditions, an apparent K_m for induction by glucose 6-phosphate was calculated to be 20 μM (34, 215, 216). Since the affinity for induction was much higher than that for transport, it is difficult to imagine that transport is directly involved in induction.

A study of the autocatalysis of *uhp* induction provides further support for an extracellular induction mechanism (34). A transport-negative bacterial culture which utilizes an intracellular induction mechanism shows a linear increase in substrate uptake versus cell mass if grown in the presence of a nonsaturating concentration of inducer. An intracellularly induced transport-positive culture under these same conditions becomes induced as the molecules of inducer are first transported into the cell, causing more transport proteins to be synthesized and so increasing uptake activity in a sigmoidal rather than a linear fashion. However, when grown in low concentrations of glucose 6-phosphate, a culture positive for the hexose phosphate transport system shows a linear increase in uptake rate versus cell mass. This lack of autocatalysis is consistent with an extracellular induction mechanism.

Physiological and Mechanistic Significance of Extracellular Induction

Speculation as to the physiological basis for extracellular versus intracellular induction suggests an explanation for some interesting observations. In enteric bacteria, such as *E. coli* and *S. typhimurium*, the substrates of extracellularly induced permease systems (Table 5) appear to have the following feature in common: the internal pools of the phosphoglycerates, tricarboxylic acids, and sugar phosphates, which either are transported by these systems or are the intracellular products of the transport processes, are normally present in appreciable concentrations in log-phase cells. Considering the fact that micromolar quantities of inducer are needed to cause induction of these systems, analogous systems which are induced intracellularly would be expected to be partially or completely constitutive in growing cultures. Consequently, cellular energy would be wasted for unnecessary protein synthesis and by leakage of solutes from the cells when the substrates are not present in the extracellular medium. Postma and Van Dam (133) drew the same conclusion from their work with Krebs cycle intermediates in the gram-negative organism *Azotobacter vinelandii*. They postulated that the inducer must be in contact with the external surface of the cell envelope in order to effect induction. As previously mentioned, aconitase mutants of the gram-positive organism *B. subtilis* (203), which cannot metabolize citrate, show constitutive citrate uptake. Willecke and Pardee (203) interpret this endogenous induction as being due to the accumulation of citrate in the cells. However, their experiments do not rule out the possibility of an efflux of citrate from the mutants and subsequent ex-

tracellular induction.

Beyond the elucidation of a unique form of regulation of protein synthesis, the study of the mechanism of extracellular induction could provide a model system for complex regulatory phenomena in higher organisms. For example, it is possible that extracellular induction in bacteria occurs by a mechanism analogous to hormonal regulation (32, 177) or to immunoglobulin induction (i.e., T-cell independent immunoglobulin M induction) (1) in animal cells. The latter processes can be thought of as interactions of extracellular molecules with membrane receptors exposed to the external cell surface. This interaction ultimately causes specific changes in the rates of protein synthesis. In bacterial extracellular induction, the inducer molecule is presumed to interact with a receptor on the external surface of the cytoplasmic membrane (the external combining site of a transmembrane protein), causing an increased rate of synthesis of new proteins (Fig. 5 and 6). Thus, the process superficially resembles the process of surface activation in animal cells. Since none of these mechanisms is understood at the molecular level, further experimentation will be required to determine whether this superficial resemblance is a reflection of common, evolutionarily related processes.

Mechanistic Studies on *uhp* Induction

The literature concerning the molecular mechanism of extracellular induction has been restricted almost exclusively to the *uhp* system in *E. coli*. This transport system derives its energy from proton cotransport (42) and does not involve a binding protein. The system maps at approximately 81.5 map units (43, 75) on the revised *E. coli* map (8). The analogous region in *S. typhimurium* maps at approximately 80 map units on the revised map for this organism.

Three classes of *uhp*-specific mutants have been isolated. Besides constitutive mutants (*uhpC*) (35, 45), two classes of negative mutants have been described. Reasoning that a negative lesion in a regulatory gene would revert occasionally to a regulation-modified phenotype, Kadner (74) mutagenized a number of *uhp*-negative mutants and selected phenotypic revertants. Some of the revertants synthesized hexose phosphate permease constitutively. Any uhp^- strain giving rise to constitutive pseudorevertants was designated *uhpR* (regulatory). Other uhp^- strains did not revert to constitutivity, and these were designated *uhpT*. The latter lesion was assumed to be in the structural gene(s) for the transport protein(s). The order of genes on the *E. coli* map appears to be *pyrE*, *uhpT*, *uhpR*, *uhpC*, *ilv* (43, 74).

Both the similarity of the *E. coli* and *S. typhimurium* genomes in the region near 80 map units and preliminary evidence from our laboratory showing approximately 50% cotransduction of *gltC* with *uhp* by bacteriophage P22 support our assumption that the fine structure map for *E. coli* will apply to *Salmonella*. In addition, Eidels et al. (40) have found that the specificity for substrates, as well as inducers, in *Salmonella* is virtually identical to that in *E. coli*.

The model which we have proposed (152) (Fig. 5) to explain extracellular induction of the *uhp* system is fully consistent with the results reported in the literature and summarized above. This model differs significantly from each

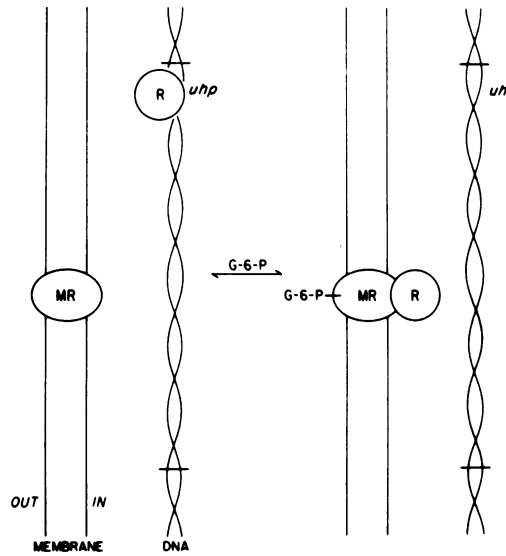

FIG. 5. *Possible mechanism for the regulation of gene expression by exogenous inducer. The model suggests an explanation for transcriptional regulation of the hexose phosphate transport system in E. coli, as well as of the phosphoglycerate transport system in S. typhimurium. Two regulatory proteins are proposed. One is a repressor protein (R), which possesses affinity for the controller region of the operon coding for the transport protein. It also binds to a transmembrane regulatory protein (MR), which possesses a glucose 6-phosphate (G-6-P) binding site on the external surface of the cytoplasmic membrane. The binding of glucose 6-phosphate to the MR protein determines its affinity for the R protein. There are no data available which distinguish this model from one in which a cytoplasmic activator protein (instead of a repressor protein) controls expression of the operon (see text). DNA, Deoxyribonucleic acid.*

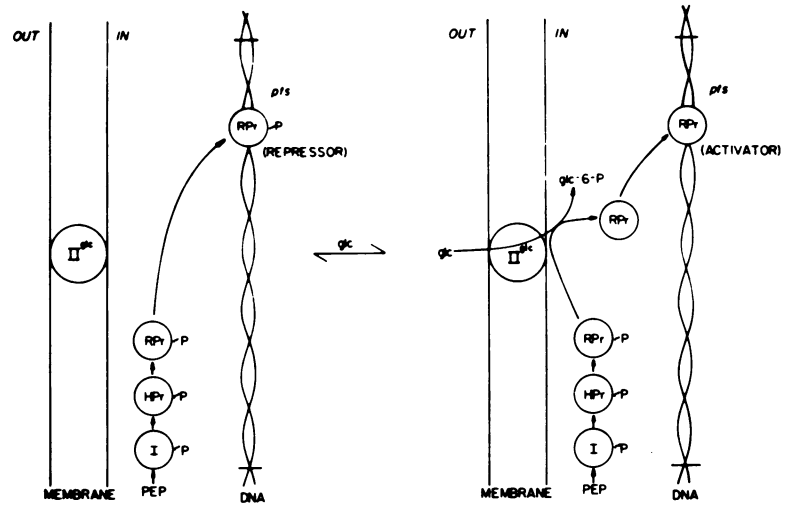

FIG. 6. *Proposed mechanism for the regulation of transcription of the pts operon in S. typhimurium. The model suggests that a regulatory protein (here designated RPr) which can be phosphorylated at the expense of phospho-HPr can interact directly with the operator region of the pts operon. Phospho-RPr may be a repressor protein, and/or free RPr may be an activator. The addition of a substrate of the PTS to the cell suspension would be expected to drain phosphate off the phosphorylated energy-coupling proteins of the PTS with concomitant phosphorylation of sugar. Hence, phospho-RPr would be dephosphorylated to RPr, and transcription of the pts operon would be induced. Abbreviations: glc, glucose; II^{glc}, enzyme II specific for glucose and methyl-α-glucoside; PEP, phosphoenolpyruvate; glc-6-P, glucose 6-phosphate; I, enzyme I; DNA, deoxyribonucleic acid.*

of the five models proposed by Dietz (34). The difference in specificity noted above for transport and induction of the *uhp* system argues in favor of two distinct active sites for these two functions. In our model we assume that the two hexose phosphate binding sites reside on two distinct proteins. The induction site is assumed to be located on the external surface of a transmembrane protein. Binding of inducer to this protein results in a conformational change which enhances its affinity for a soluble cytoplasmic regulatory protein, here presumed to be a repressor of the *uhp* operon. Direct interaction of the genome with a membrane protein (as proposed by Dietz) is considered to be unlikely since this would require that the deoxyribonucleic acid be in specific contact with the membrane wherever an extracellularly induced operon is present.

Induced Synthesis of Phosphotransferase System Proteins

Recently, we characterized the induced synthesis of protein constituents of the PTS in *S. typhimurium* (141). Some of these enzymes (enzyme I, HPr, enzyme II^{glc}, and enzyme II^{man}, coded for by the *ptsI*, *ptsH*, *glu*, and *manA* genes, respectively) appeared to be induced extracellularly. The following observations were made (141). (i) Induction was dependent on the extracellular sugar substrate of the PTS, as well as the enzyme II specific for the inducing sugar. Genetic loss of the enzyme II resulted in a noninducible phenotype with respect to that particular sugar substrate. (ii) Induced synthesis of all four proteins was dependent on cyclic AMP and a functional cyclic AMP receptor protein. In the absence of either agent, the rate of protein synthesis was basal or subbasal. (iii) Several extracellular substrates of the PTS induced expression of the *pts* operon; fewer induced the *glu* and *manA* genes. However, in both cases the best inducers appeared to be the most rapidly transported substrates of the PTS, and poor substrates were poor inducers. (iv) Loss of either enzyme I or HPr activity as a result of point mutations in the structural genes for these proteins gave rise to high-level constitutive synthesis of the remaining three proteins under consideration. Deletion of the *pts* and *crr* genes also resulted in constitutive synthesis of enzyme II^{glc} and enzyme II^{man}. (v) Although methyl-α-glucoside, a non-metabolizable substrate of enzyme II^{glc}, did not induce synthesis of the PTS enzymes in a wild-type strain of *S. typhimurium*, induction of the *pts* operon by this sugar did occur in a leaky enzyme I mutant, strain *ptsI17* (154; Apperson, unpublished data). These results have led to the postulation of a mechanism

which involves phosphorylation of a regulatory protein which can interact with the deoxyribonucleic acid to regulate transcription (Fig. 6). As proposed, phosphorylated RPr is a transcriptional repressor (negative control [Fig. 6, left]) and/or free RPr is an activator (positive control [Fig. 6, right]). A third possibility, that phosphorylated HPr acts as a repressor, has not been eliminated.

CONCLUDING REMARKS

The foregoing discussion has focused on mechanistic and regulatory aspects of bacterial transport systems. At least five distinct energy-coupling mechanisms characterize bacterial permeases (Fig. 1). A carbohydrate may cross the membrane in an energy-independent process or by a concentrative transport mechanism involving the expenditure of chemical or chemiosmotic energy. The substrate may remain unaltered, or it may be phosphorylated during entry into the cytoplasmic compartment. The diversity of energy-coupling mechanisms may or may not reflect the types of transmembrane translocation mechanisms. Insufficient evidence is presently available for us to delineate the steps which comprise the translocation process. However, two distinct mechanisms of transport can be envisioned. First, binding protein-dependent systems may translocate substrates across the membrane by a pore-type mechanism (Leonard et al., in press). A substrate may shuttle from an external binding site through a substrate-induced stereospecific pore to a distinct binding site on the cytoplasmic surface of the membrane. The specificities of such systems may lie primarily in the two solute combining sites rather than the transmembrane channel. Transport of solutes by this mechanism may involve minimal conformational changes, and hence these systems may be relatively insensitive to the physical state of the hydrophobic environment in which the permease protein is imbedded. Among the best-characterized transport systems which may function by such a mechanism are the maltose, succinate, and histidine permeases of *E. coli* and *S. typhimurium* (104; Leonard et al., in press).

Active transport systems which utilize a chemiosmotic source of energy may utilize a carrier-type translocation mechanism involving a single substrate binding site. The evidence concerning the number of sugar binding sites on the lactose permease remains equivocal. However, most of the evidence favors a one-site model, where the two classes of substrates either bind to the permease with differing affinities or induce different protein conformations. (The notion of substrate-induced energy coupling is discussed above.) Assuming a one-site model, available kinetic data (72, 73) appear to be most easily interpreted in terms of a carrier-type model. Indeed, as expected from this prediction, transport systems which function by proton symport appear to be more sensitive to the state of the membrane matrix (Leonard et al., in press). Furthermore, although these systems catalyze accelerative exchange transport, the same is not true of permeases which utilize periplasmic binding proteins. Exchange transport may be a characteristic feature of permeases which function by a carrier-type mechanism.

The enzymes II of the PTS clearly function as the sugar recognition and translocating components of the system. The available evidence favors a mechanism which, like the lactose permease, depends upon a single substrate binding site which can "flip" from one orientation (a single binding site on the external surface of the membrane) to another conformation (a sugar phosphate binding site on the cytoplasmic surface of the membrane). The demonstration of exchange group translocation which, in contrast to unidirectional group translocation, may involve the simultaneous cooperation of two protein subunits, is fully consistent with such a mechanism. It is worth noting that such a mechanism might incorporate features of the channel-type mechanism suggested for the binding protein-dependent systems, as well as the carrier-type mechanism proposed for the lactose permease.

Although evidence bearing on the mechanism of facilitated diffusion is not available, the possible substrate-induced conversion of active and group translocating transport systems to energy-uncoupled carriers suggests that glycerol may cross the membrane by a related process. Other energy-independent transport proteins, such as the bacterial porins which exhibit low specificity for the transported substrate, undoubtedly function by pore-type mechanisms.

Just as a multiplicity of transport and energy-coupling mechanisms are becoming recognized, a variety of regulatory constraints controlling permease function and synthesis appear to be operative. Evidence for five distinct mechanisms of solute permeation control is discussed above, as are three different mechanisms of permease induction. The details of these processes remain poorly defined, and the elucidation of their mechanistic features will be challenging problems for biologists. A conceptual understanding of the smooth, coordinated functioning of the

different constituents of the cellular machinery under conditions of homeostasis or in response to environmental change remains the ultimate goal.

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