MICROBIOLOGICAL REVIEWS, Sept. 1985, p. 232–269
0146-0749/85/030232-38$02.00/0

Vol. 49, No. 3

Phosphoenolpyruvate:Carbohydrate Phosphotransferase System of Bacteria

P. W. POSTMA[1]* AND J. W. LENGELER[2]

Laboratory of Biochemistry, University of Amsterdam, 1018 TV Amsterdam, The Netherlands,[1] and Fachbereich Biologie/Chemie, Universität Osnabrück, D-4500 Osnabrück, Federal Republic of Germany[2]

INTRODUCTION

In contrast to most single cells of a eucaryotic organism, whose surroundings are kept relatively constant by the organism itself, free-living unicellular bacteria must be able to adapt rapidly. Therefore, one of the most challenging problems for a bacterial cell is to detect the frequent changes in the environment and to regulate and integrate its metabolism in accordance with such changes. In bacteria, the transport systems or parts of them are the sense organs for chemical stimuli. Since these systems are also the pacemakers for all peripheral catabolic pathways, their functions are of pivotal importance in cellular adaptation.

Bacteria have evolved a variety of mechanisms by which

* Corresponding author.

solutes are transported through membranes. The corresponding transport systems, localized primarily in the cytoplasmic membrane, recognize groups of structurally related compounds and are able to catalyze their translocation across the membrane. If this carrier-mediated translocation is not coupled to metabolic energy (i.e., occurs by facilitated diffusion), the solute moves only along preexisting gradients and is not accumulated on either side of the membrane. In many cases, however, the solute is accumulated against an electrochemical gradient in an energy-dependent process (active transport). Active transport in bacteria is energized either by means of chemical gradients of (proton or sodium) ions in the form of symport, antiport, or uniport (237, 238) or by as yet unidentified high-energy compounds. All carrier-mediated active transport systems or facilitated diffusion systems have one property in common: the solute is translocated through the membrane in an unchanged form (107, 135, 394).

The present review deals with a different type of bacterial transport system, the phosphoenolypyruvate-dependent carbohydrate:phosphotransferase system (PTS). This system catalyzes the transport and concomitant phosphorylation of a series of carbohydrates (PTS carbohydrates), resulting in the intracellular accumulation of the corresponding carbohydrate phosphates (group translocation). Each carbohydrate phosphate invariably is the first intermediate in the catabolism of the carbohydrate, and this metabolism starts by uptake through the PTS, thus providing a tight linkage between uptake and subsequent metabolism. No free carbohydrate is found in the cell (unless intracellular phosphatases have hydrolyzed the carbohydrate phosphate). In that respect the PTS differs from a transport system coupled to a kinase (such as that for glycerol or galactose), although formally a carbohydrate phosphate results from both processes.

The PTS consists of a number of cytoplasmic and membrane-bound proteins, each of which can exist in the phosphorylated and the nonphosphorylated state. It is one of the few transport systems whose components, including some of the membrane-bound ones, have been purified. A major advantage of this system is the fact that during purification the nonvectorial phosphorylation activity of the PTS can be measured directly by following the conversion of its substrates to the corresponding phosphate esters without the necessity of using sealed membrane vesicles. To measure the complete vectorial process, however, the transport of the solutes into or out of closed vesicles must be followed.

It is the purpose of this review to discuss new and recent developments in our understanding of this highly sophisticated system and its multiple biological functions as a transport system for numerous carbohydrates, as a chemoreceptor system in chemotaxis toward carbohydrates, and as a pacemaker regulatory system for peripheral catabolic pathways. Recent results on regulatory phenomena such as the glucose effect, catabolite and transient repression, catabolite inhibition, inducer exclusion, and diauxie are rediscussed in view of a previously formulated hypothesis which assigns an important regulatory role in such processes to the phosphorylation state of one or several of the PTS proteins.

THE PTS: AN OVERVIEW

The PTS was discovered in cell extracts of *Escherichia coli* approximately 20 years ago by Kundig et al. (182), as a system which catalyzes the phosphorylation of a number of carbohydrates with phosphoenolpyruvate (PEP) as the phosphoryl donor. Several protein fractions, both soluble and membrane bound, were required to catalyze the overall reaction (see Fig. 1). At about the same time, a series of mutants of *Staphylococcus aureus* which were either unable to grow on a single carbohydrate or had lost the capacity to grow on a variety of carbohydrates were described by Murphey and Rosenblum (244, 245), and by Egan and Morse (89–91). In both cases, a point mutation was responsible for the phenotype and seemed to affect uptake and phosphorylation of the carbohydrates and induction of the corresponding metabolic pathways. Egan and Morse considered the pleiotropic phenotype of their mutants as evidence in favor of the common carrier mechanism postulated by Kepes (164). Further biochemical and genetic analysis of such mutants of *E. coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *S. aureus* soon revealed that for many carbohydrates the uptake process involves translocation through the membrane, concomitant phosphorylation, and accumulation in the cytoplasm in the form of carbohydrate phosphates. Furthermore, it became clear that the same proteins must be involved in transport and phosphorylation (278).

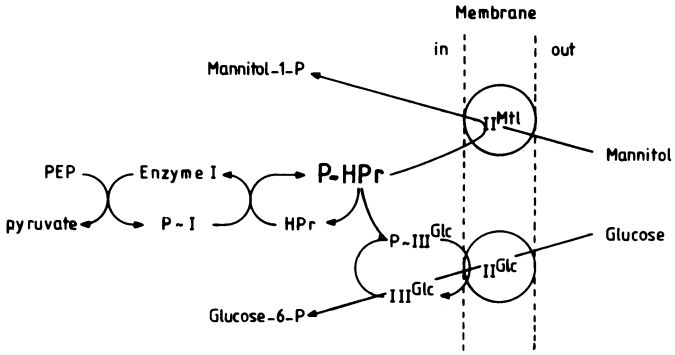

FIG. 1. The PTS. Enzyme I and HPr are the general PTS proteins. Of the many different enzymes II, only two are shown. II^{Mtl} is specific for mannitol, and II^{Glc} together with III^{Glc} is specific for glucose. P~I, P~HPr and $P\sim III^{Glc}$ are the phosphorylated forms of enzyme I, HPr, and III^{Glc}, respectively.

In general two proteins, enzyme I and heat-stable protein (HPr), are required for the first step in transport and phosphorylation of all PTS carbohydrates, resulting in the phosphorylation of HPr at the expense of PEP (Fig. 1). It became clear that the pleiotropic, carbohydrate-negative mutants, including some described before the discovery of the PTS (17, 87), were defective in enzyme II or HPr or both, but not in a general carrier. Substrate specificity of the system, however, resides in the membrane-bound enzymes II, each of which can recognize a series of structurally related carbohydrates. During translocation of a substrate through the membrane, the phosphoryl group, which was transferred from PEP through the intermediate of phospho-enzyme I to HPr (Fig. 1), is now transferred from phospho-HPr to the substrate, probably by means of a phospho-enzyme II intermediate. Consequently, loss of any type of enzyme II by mutation affects only the transport and phosphorylation of those substrates that are taken up via this enzyme II, but does not affect the uptake of all other PTS carbohydrates.

Before discussing in detail the different components of the PTS, the reactions they catalyze, and the genes involved, a few general remarks should be made on the various forms of the PTS found in a single bacterium and in different organisms.

(i) In no case analyzed thus far are the general PTS proteins enzyme I and HPr involved directly in the translocation of substrates through the membrane. Their role is restricted to phosphoryl group donation and indirectly to the regulation of other transport systems or enzymes. In a few organisms (*Rhodospirillum rubrum*, *Rhodopseudomonas sphaeroides*, and *Pseudomonas aeruginosa*), enzyme I and HPr are not distinct proteins, but rather a single protein fulfills both roles (47, 88, 321, 334).

(ii) The substrate-specific enzymes II do not necessarily consist of a single membrane-bound polypeptide, but may be composed of more than one protein (184). In some cases, an additional cytoplasmic protein, positioned between HPr and the membrane-bound protein of such an enzyme II complex, participates in carbohydrate phosphorylation (242, 327, 346, 351). This soluble phosphoryl carrier is referred to as enzyme III hereafter. Owing to appreciable hydrophobicity, the interaction of some enzymes III with their membrane-bound enzyme II is relatively strong and may resemble membrane-bound enzymes III (76). In this review we will restrict the name enzyme II to the integral membrane proteins which are responsible for the substrate specificity of the corresponding PTS activity. These proteins span the membrane and catalyze the binding and translocation of the substrates through the membrane.

To distinguish the enzymes II, abbreviated superscripts are used to define carbohydrates, so that II^{Glc}, II^{Man}, and II^{Lac} refer to the enzymes II that phosphorylate glucose, mannose, and lactose, respectively. In contrast to the general PTS protein HPr, enzymes III interact in general with only one or a few enzymes II, according to which they will be named. Finally, the term glucose-PTS or fructose-PTS, e.g., will designate the sum of all molecules involved in the PEP-dependent translocation and phosphorylation of glucose or fructose, respectively.

(iii) A particular carbohydrate might be a PTS substrate in one organism but not in another organism. For instance, lactose is transported in *S. aureus* by enzyme II^{Lac} (242) but by a proton symport system in the family *Enterobacteriaceae* (135). Furthermore, the same strain (e.g., strains of *K. pneumoniae* growing rapidly on lactose) may have a plasmid-encoded II^{Lac} in addition to the proton symport system encoded on the chromosome (see reference 127).

It is obvious that many variations on the PTS theme exist. This review will analyze the properties of the PTS both in the gram-negative enteric bacteria *E. coli* K-12, *Salmonella typhimurium*, and *K. pneumoniae*, which have been studied most extensively by biochemical and genetic methods, and in several gram-positive organisms. Various aspects of the PTS have been reviewed previously (59, 81, 132, 136, 171, 208, 278, 295, 313, 323).

COMPONENTS OF THE PTS

General, soluble proteins enzyme I and HPr

The uptake of all PTS carbohydrates is dependent on the constitutive activity of the two general PTS proteins, enzyme I and HPr. The general reaction scheme for all PEP-dependent phosphotransferase systems is the following:

$$\text{PEP} + \text{enzyme I} \rightleftharpoons \text{phospho-enzyme I} + \text{pyruvate} \quad (1)$$

$$\text{phospho-enyzme I} + \text{HPr} \rightleftharpoons \text{enzyme I} + \text{phospho-HPr} \quad (2)$$

$$\text{phospho-HPr} + \text{enzyme II} \rightleftharpoons \text{HPr} + \text{phospho-enzyme II} \quad (3)$$

or

$$\text{phospho-HPr} + \text{enzyme III} \rightleftharpoons \text{HPr} + \text{phospho-enzyme III} \quad (3a)$$

$$\text{phospho-enzyme III} + \text{Enzyme II} \rightleftharpoons \text{enzyme III} + \text{phospho-enzyme II} \quad (3b)$$

$$\text{phospho-enzyme II} + \text{carbohydrate}_{out} \rightarrow \text{enzyme II} + \text{carbohydrate phosphate}_{in} \quad (4)$$

The first step in the phosphotransfer reaction sequence catalyzed by the PTS is the phosphorylation of the general protein enzyme I (reaction 1). The phosphoryl group in phospho-enzyme I is linked to the N3 position in the imidazole ring of a histidine residue. Enzyme I has been purified from *E. coli* (296, 388), *Salmonella typhimurium* (391), *Streptococcus faecalis* (10) and *Mycoplasma* (150). Its overall amino acid composition is not particularly hydrophobic. The preparations were found to be homogeneous by isoelectric focusing data, sedimentation velocity measurements, and polyacrylamide gel electrophoresis under native or denaturing conditions in which the enzymes migrate as a single band. For the monomer from *E. coli* and *Salmonella typhimurium*, molecular weights (MWs) in the range of 57,000 to 70,000 have been reported, depending on the conditions used. Cloning of the *ptsI* gene of *E. coli* K-12 and its expression in a maxicell system has shown that this is the structural gene for enzyme I and is sufficient for its synthesis. According to the cloning data, the MW of the product of the gene *ptsI* has a maximal value of 68,000. Complementation of an enzyme I-thermosensitive mutant by various plasmids that contained the *ptsI* gene and expressed one polypeptide of MW 68,000 restored the Pts^+ phenotype (75). Although similar in their biochemical properties and fully active in heterologous in vitro exchange tests, the enzymes from *Salmonella typhimurium* and *E. coli* K-12 have a slightly different immunochemical reactivity (226), whereas, as expected, the differences between these enzymes I and those from gram-positive bacteria are large (10, 233).

The monomeric subunits of enzyme I are catalytically inactive. Dimers are formed in a reversible monomer-dimer association reaction. Kinetic studies with the purified components indicate furthermore that apparently only the enzyme I dimer can be phosphorylated (180, 235, 389). Conflicting results have been reported on the number of phosphoryl groups incorporated into enzyme I during this process. Although Misset and Robillard (236) found only one phosphoryl group per enzyme I dimer in the *E. coli* enzyme, Weigel et al. (389, 391) reported two phosphoryl groups per mole of enzyme I dimer for *Salmonella typhimurium*. This discrepancy cannot be resolved at present.

Phospho-enzyme I and the soluble phosphoproteins of the PTS have the highest phosphoryl transfer potential (about twice that of adenosine triphosphate [ATP]) of all known naturally occurring phosphoryl compounds (the apparent equilibrium constant for the overall reaction [reactions 1 and 2] has been estimated to be 11 ± 7.7 [389]). This potential is conserved until very late in the overall reaction scheme, probably up to the formation of phospho-enzyme II. Phospho-enzyme I can transfer its phosphoryl group back to pyruvate to form PEP, but also to other keto acids and their structural analogs such as bromopyruvate, several phosphono-derivates, or β-hydroxypyruvate (330). Of these analogs, 2-ketobutyrate has recently been claimed to play an essential role in the regulation of the PTS (67, 70), whereas bromopyruvate and vinylglycolate are analogs which (the

latter in the form of its derivate 2-keto-butenoate) inhibit the enzyme irreversibly (142, 331, 378). In addition to the histidyl residue directly involved in phosphoryl transfer, a nearby sulfhydryl group seems to be involved in electron donation-acceptance, explaining the extreme sensitivity of enzyme I to sulfhydryl reagents (295).

Incubation of enzyme I with 3-bromopyruvate showed (142) that first one essential cysteine residue is alkylated at the active site of the enzyme I dimer without causing any loss of enzymatic activity in the PEP-pyruvate phosphoryl group exchange, the phosphoryl group transfer from phospho-enzyme I to HPr, or dimerization of alkylated monomers to the active form. Alkylation of a second cysteine residue causes complete inactivation. Simultaneous addition of substrate and product protects against this inactivation of the catalytic site, especially if oxalate (the product with the highest affinity) is used. In the presence of oxalate, 2 mol of labeled bromopyruvate is incorporated per dimer without a loss of activity, and only incorporation of a third mole results in inactivation. Hoving et al. (142) explained the data by assuming an active enzyme I dimer possessing two nonidentical subunits. This is hard to reconcile with the finding that there is only one structural gene for enzyme I (see below). Whether this asymmetry is related to the finding by the same group (236) that only one phosphoryl group is incorporated by an enzyme I dimer remains to be shown.

The main function of phosphorylated enzyme I is, of course, the transfer of its phosphoryl group to the second general protein of the PTS, the histidine-containing phosphocarrier protein HPr, to form phospho-HPr according to reaction 2. Enzyme I is restored to its original state and thus can be considered an enzyme in the classic sense. Since one of its natural products is the protein HPr, however, it may also be considered a phosphoryl carrier protein or a PEP-dependent protein kinase.

HPr, the second general PTS protein involved in the phosphoryl transfer reaction sequence, is a small protein. The amino acid sequence of the protein from *Salmonella typhimurium* has been determined previously (390), but has not yet been confirmed at the deoxyribonucleic acid (DNA) level. According to the published data, it contains 84 amino acids (MW, 9,017) with two histidine residues, at positions 15 and 75. The phosphoryl group derived from phospho-enzyme I is attached solely to the N-1 position of the imidazole ring of His-15, a phosphorylation which does not require divalent metal ions. Thus His-15 must be part of the active site. If during the purification of this protein, originally called heat-stable protein, a heat step is included, two less active forms, HPr1 and HPr2, originate, probably by deamidation (390). HPr from *E. coli* K-12 and *K. pneumoniae* seem very similar, if not identical, to the HPr from *Salmonella typhimurium* (13, 85, 390; H. De Reuse, A. Roy, and A. Danchin, Gene, in press). Since for other homologous proteins of the family *Enterobacteriaceae* up to 11% amino acid substitution and up to 25% base pair substitutions are observed (34, 141, 254, 364), this conservation of the HPr primary sequence could indicate strict requirements for its structure or functions or both. Interestingly, none of the enterobacterial HPr's seems to contain tryptophan, tyrosine, or cysteine (13, 390). Contrary to an early report, all HPr activity is found intracellularly; none is found in the periplasm.

Other HPr's that have been purified or sequenced are those of *Mycoplasma capricolum* (MW, 9,500) (149), and of *S. aureus* (MW, 7,685) (27, 352). The proteins from gram-positive and gram-negative bacteria differ considerably. Thus, of the 84 amino acids of the enterobacterial HPr (lacking tyrosine), only 13 are identical to the 70 amino acids of HPr from *S. aureus* (containing three tyrosines). Of these 13 amino acids, 12 occur in the first 31 residues of both proteins, clustered around His-15, part of the active site. Despite these differences in the primary sequence, ^{1}H-nuclear magnetic resonance studies with HPr's from various bacteria have indicated that major similarities in the secondary structures exist, e.g., the location of His-15 on a protrusion rather than a cleft of the protein (160). These similarities are also supported by the observation that enterobacterial enzyme I can phosphorylate, although at a low level, the HPr from *S. aureus* and vice versa (13, 160).

HPr's from several streptococci (*Streptococcus pyogenes*, *Streptococcus lactis*, and *Streptococcus faecalis*) and from *Bacillus subtilis* (10, 160, 223) have almost the same MW as that from *S. aureus* (MW, 7,685), whereas *Streptococcus mutans* HPr has an MW of 17,000 (233). The HPr's from these gram-positive organisms can be phosphorylated in two ways: in a PEP- and enzyme I-dependent reaction at His-15 and in an ATP-dependent reaction at a seryl residue (acid-stable bond). The latter process, which seems to be involved in the regulation of HPr activity, is discussed below.

Enzymes III: soluble enzyme II-specific phosphocarrier proteins

In a number of cases, phospho-HPr transfers its phosphoryl group directly to the substrate-specific, membrane-bound enzyme II of the PTS (reaction 3). In some cases, however, transfer occurs instead to a third soluble protein called enzyme III (see Table 1).

Transport and phosphorylation of glucose and several of its nonmetabolizable analogs (e.g., methyl α-glucoside) in the family *Enterobacteriaceae* requires an enzyme III^{Glc} (181, 184, 326). It acts as a phosphoryl carrier from phospho-HPr to the membrane-bound II^{Glc} (reaction 3b), coded for by the gene *ptsG*. The III^{Glc} is also required for the uptake and phosphorylation of sucrose in *E. coli* K-12 (200). Plasmids containing genes coding for a II^{Scr} and two additional proteins allow strains of *E. coli* K-12 to grow on sucrose only when an active III^{Glc} is present. In the absence of III^{Glc}, no sucrose uptake into whole cells and no PEP-dependent phosphorylation of sucrose in an in vitro test system is observed.

III^{Glc} from *Salmonella typhimurium* has been isolated and purified to apparent homogeneity by different methods (228, 346). Furthermore, the corresponding structural gene, *crr*, of *E. coli* and *Salmonella typhimurium* has been cloned (46, 75, 230, 249), amplified, and sequenced (249). The calculated MW of its 169 amino acids residues is 18,556. The amino acid sequence deduced from an open reading frame of the cloned *crr* gene is in perfect agreement with the partial NH_2-terminal sequence determined by Edman degradation of pure III^{Glc}. Under denaturing conditions the pure monomer has an apparent MW of 20,000. Similarly to HPr, it lacks cysteine, tyrosine, and tryptophan. Intact III^{Glc} accepts one molecule of phosphate from PEP, and the phosphoryl group is linked at the N-3 position of the imidazole ring of His-91, one of the two histidyl residues found in the molecule (86, 228). Reaction 3a is completely reversible. Characteristics of III^{Glc} are a remarkable thermal stability and a strong tendency to form dimers, trimers, or hexamers, a property lost in certain *crr* mutants (347). A previous report (181) that the native enzyme consists of three to four subunits (MW, 5,000

to 6,000) in which the phosphoryl bond is an acyl phosphate and which copurifies with a strong hexose 6-phosphatase activity could not be confirmed.

On the basis of different electrophoretic mobilities on native gels, two forms of III^{Glc} were identified. One form, III^{Glc}_{fast}, was derived from a second, III^{Glc}_{slow}, by cleavage of the seven NH_2- terminal amino acids (228, 249, 346). This processing increases the apparent MW under denaturing conditions (from 19,000 to 21,000). The effect is most probably due to an altered binding of sodium dodecyl sulfate to the protein and has been observed previously, most clearly in the multiple forms of the methyl-accepting chemotaxis proteins (for a review, see reference 42). The processing, although not affecting the phosphorylation of III^{Glc}_{fast} by phospho-HPr (reaction 3a), decreases the phosphoryl group transfer from phospho-III^{Glc}_{fast} to II^{Glc} (reaction 3b) to 2 to 3% of the value found with phospho-III^{Glc}_{slow} (228). A similar effect is observed after derivatization of the molecule at the NH_2-terminal end by fluorescein-5-isothiocyanate (144). These results suggest that the NH_2-terminal part of III^{Glc} is important for its interaction with II^{Glc}.

Meadow and Roseman (228) ascribed the conversion of III^{Glc}_{slow} to III^{Glc}_{fast} to the presence of unspecific proteases present in cell extracts, although a physiological role of the derivatized III^{Glc} in regulation was not excluded. Nelson et al. (249) observed that a plasmid containing the structural gene *crr* for III^{Glc} codes in the maxicell system of Sancar and Rupp for two proteins with apparent MWs of 20,000 and 21,000. Immunologically, both forms are closely related and may correspond to the slow and fast forms observed by Meadow and Roseman. The existence of different forms of III^{Glc} is further confirmed by new procedures developed to detect acid-stable and -labile forms of the different PTS proteins on native isoelectric focusing gels (225). These procedures also allow some differentiation between N1- and N3-phosphohistidine residues.

Unexpectedly, membranes from mutants of *Salmonella typhimurium* with a deletion or Tn*10* insertion in the *crr* gene still have III^{Glc}-like activity. Such membranes phosphorylate methyl-α-glucoside in a PEP-, enzyme I-, and HPr-dependent reaction, and this activity is abolished by antibodies against purified soluble III^{Glc} (347). The activity is dependent, furthermore, on intact II^{Glc}, as was also shown in transport assays. Mutants which lack soluble III^{Glc}, owing to a mutation in the *crr* gene, still have about 20% of the activity of a wild-type strain. Possibly a second gene exists, coding for a III^{Glc}-like protein.

Antibodies have been used to detect III^{Glc}-related proteins in other members of the family *Enterobacteriaceae* such as *Klebsiella* spp. (346), but also in *Vibrio parahaemolyticus* (178, 346). A glucose-PTS in this organism was found to contain four components, including a III^{Glc}-like protein (MW, 30,000 on Sephadex gels). Some mutants unable to grow on glucose and trehalose were found to lack this protein (179). No such material has been detected in more distantly related bacteria, but this might be due to growth under noninducing or repressing conditions.

By means of gels in which it is possible to detect PEP-dependent protein phosphorylation, further soluble proteins closely related to the PTS have been detected in cell extracts of *E. coli* K-12 and *Salmonella typhimurium* (225, 386). These phosphoproteins, however, are not found under all growth conditions and differ in this respect from the constitutively expressed enzyme I, HPr, and III^{Glc}. After pregrowth on fructose, two or possibly three additional proteins (relative MW [M_r], 40,000, 13,000, and 8,000) were found in cell extracts compared with extracts from cells pregrown on glycerol. The largest of these soluble proteins has several properties which indicate that it could be a III^{Fru}. Besides being inducible by fructose in the growth medium, the stability of its phosphoryl bond is similar to that of the *N*-3-phospho-histidine (N3-phospho-His) of enzyme I and III^{Glc}, but not to the N1-phospho-His of HPr. Furthermore, an active HPr molecule is not necessary for the transfer of the phosphoryl group from phospho-enzyme I to III^{Fru}. One of the small molecules (M_r, 8,000) is induced by D-fructose and apparently substitutes for HPr in this reaction. It is probably identical to a protein previously called FPr (128, 333) or "pseudo-HPr" (59). A protein with properties similar to those of the III^{Fru} has been described originally for *K. (Aerobacter) aerogenes* (379). This protein was named K_m-factor, since it apparently decreased the Michaelis constant (K_m) of a D-fructose-specific enzyme II for fructose. In view of the results obtained with *E. coli* and *Salmonella typhimurium*, the most likely explanation is that the factor did not alter the K_m of an enzyme II, but rather that there are different enzymes II which can catalyze D-fructose uptake. One is the constitutive II^{Man}, which is coded for by the gene *ptsM*, has a low affinity for fructose (K_m, 10 mM), and generates fructose 6-phosphate. The other is the II^{Fru} of the fructose-PTS, coded for by the gene *fruA*. It has a high affinity for fructose (K_m, 10 μM), generates fructose 1-phosphate, and requires the K_m factor for its activity. Names such as K_m factor, FPr, and Pseudo-HPr have been used by different groups of workers to designate proteins related to the fructose-PTS (59, 128, 333, 379, 385, 386). They should be used cautiously, since neither their relation to the proteins described above nor their exact function as part of the fructose-PTS is known.

Preliminary biochemical or genetic evidence for the presence of other enzymes III in members of the family *Enterobacteriaceae* has been published recently. According to Sarno et al. (339) a III^{Gut} (M_r, 16,000) in *Salmonella typhimurium* is involved in the phosphorylation of D-glucitol (sorbitol) to D-glucitol 6-phosphate by the II^{Gut} (M_r, 45,000), coded for by the gene *gutA* (191). The evidence, however, is neither sufficient to prove the existence of a third structural gene, *gutB*, in the *gut* operon, postulated to code for this protein, nor to rule out the possibility that the small polypeptide is a degradation product of II^{Gut}. Waygood et al. (386) also found in cell extracts of *E. coli* and *Salmonella typhimurium* a protein (M_r, 13,000 and 14,000, respectively, and apparently containing an N3-phospho-His) that can be phosphorylated by PEP and that stimulates glucitol phosphorylation. It might thus be identical to the III^{Gut} postulated by Sarno et al. except for its inducibility by growth on fructose.

Waygood et al. (386) also reported the existence of a soluble III^{Man} (M_r, 33,000), which appears to be synthesized constitutively. It is phosphorylated at the expense of phosphoenolpyruvate to form an N3-phospho-His and interacts with II^{Man} but not with II^{Glc}. The system reconstituted from partially purified II^{Man} and III^{Man} has the substrate specificity reported for the mannose-PTS coded for by the gene locus *ptsM* (see Table 2).

In *S. aureus*, lactose and several structurally related carbohydrates are accumulated as galactoside 6-phosphates in a process catalyzed by a lactose-PTS (136, 242, 351–353). Besides the general proteins enzyme I and HPr, two proteins inducible by growth on lactose are required: a soluble III^{Lac} and a membrane-bound II^{Lac}. The III^{Lac} has been purified to homogeneity (M_r of monomer, 11,000 to 12,000). Its active

form seems to be a trimer of identical subunits (133). Each subunit contains four histidyl residues, only one of which is phosphorylated, at the expense of PEP, to form an N3-phospho-His bond. The unusual trimeric structure is possibly an artifact, observed only at high protein concentrations and in the absence of membrane phospholipids (76, 357). The monomer seems to contain a hydrophobic stretch of amino acids. In an aqueous environment and at high protein concentrations, trimers form in which the hydrophobic parts of nonphosphorylated subunits are mutually shielded. During the induction of new molecules within the cell, each monomer is phosphorylated immediately and enters the membrane to form a complex with II^{Lac}. Both ^1H-nuclear magnetic resonance and immunological studies with phosphorylated and nonphosphorylated III^{Lac} strongly support such drastic conformational changes and the predicted alteration in the hydrophobicity of III^{Lac} (76, 357).

A rapidly increasing number of soluble enzymes III have been reported (Table 1): III^{Mtl} in *S. aureus* (107), III^{Gnd} (gluconate [26]), III^{Fru} (fructose [111]), and III^{Scr} (sucrose [233]) in streptococci, and III^{Gal} (galactose [53]) and III^{Xtl} (xylitol [214]) in lactobacilli. Thus it appears as if, in gram-positive bacteria, soluble enzymes III are the general rule. The III^{Gal} from *Lactobacillus casei* has an MW of 35,000, similar to III^{Lac} from *S. aureus*. A III^{Xtl} from the same organism, however, consists of only 109 amino acids (M_r, 11,000 to 12,000). This molecule readily absorbs to octyl-Sepharose and thus resembles many of the PTS proteins. It is not related immunologically to III^{Lac} or III^{Gal} from the same organism and is inducible by pentoses only. Interestingly, the molecule is always isolated in the phosphorylated form. Treatment with alkaline phosphatase converts the molecule to a more basic form with an altered electrophoretic mobility (214). The molecule contains five histidine residues, one of which could be phosphorylated. It remains to be shown whether the phospho bond in the isolated III^{Xtl} is an N3-phospho-His bond or another stable form.

TABLE 1. Enzymes III of the PTS

Enzyme III	Organism	Reference
III^{Glc}	*E. coli-Salmonella typhimurium*	228, 327, 346
III^{Glc}	*V. parahaemolyticus*	178, 346
III^{Man}	*E. coli-Salmonella typhimurium*	386
III^{Fru}	*E. coli-Salmonella typhimurium-K. pneumoniae*	128, 386
III^{Gut}	*E. coli-Salmonella typhimurium*	339, 386
III^{Lac}	*S. aureus*	76, 133, 242
III^{Mtl}	*S. aureus*	106
III^{Fru}	Streptococci	111
III^{Gnd}	Streptococci	26
III^{Scr}	Streptococci	233
III^{Gal}	Lactobacilli	53
III^{Xtl}	Lactobacilli	214

Integral, membrane-bound PTS proteins

Membranes from members of the family *Enterobacteriaceae* contain many different substrate-specific enzymes II, most of which are inducible by pregrowth of the cells on a particular carbohydrate. As indicated above, the enzymes II are best named according to their main substrate, which for inducible systems is invariably also the inducer (Table 2). On the basis of biochemical and genetic data, 13 different enzymes II have so far been identified in the enteric bacteria. Two of these (II^{Scr} and II^{Lac}) are coded for by genes located on metabolic plasmids. As a general rule, enzymes II have a broad substrate specificity such that almost any substrate or substrate analog can be taken up by more than one enzyme II. This complicates their analysis considerably. In mutants which lack all except one enzyme II for a given carbohydrate, clear-cut kinetic studies can be done.

Recently, enzyme II^{Mtl} from *Salmonella typhimurium* has been purified to homogeneity, the first PTS membrane protein for which this has been achieved. It has been characterized extensively by reconstitution into liposomes, by determining the intramembrane topology, and by sequencing its structural gene (145–148, 187, 314). On the basis of enzymatic tests and a careful genetic analysis of different mutants with defects in II^{Mtl}, it had been predicted that II^{Mtl} in *E. coli* K-12 consists of one protein or a complex of identical subunits (191, 202). II^{Mtl} was purified by hydrophobic chromatography on hexyl-agarose after extraction of the enzyme from the membranes with sodium deoxycholate and elution from the columns by Lubrol PX. Its true MW, calculated from the complete DNA sequence of the molecule, is 67,893. This contrasts with a value of 60,000 deduced from sodium-dodecyl sulfate-gel electrophoresis, indicating an atypical mobility of the membrane protein on such gels. There was no indication for the existence of a signal peptide sequence at the NH_2-terminal end of the molecule. Experiments with hydrophilic cross-linking reagents failed to reveal any multimer in the membrane, although gel filtration experiments with the native enzyme in the presence of Lubrol PX indicated an apparent MW in excess of 700,000. Thus, the real quaternary structure of II^{Mtl} in the membrane remains to be shown. Labeling studies of II^{Mtl} in inverted cytoplasmic membrane vesicles with [^{14}C]*N*-ethylmaleimide suggest that under these conditions the native enzyme is composed of two identical subunits linked by a disulfide bridge (304). Recently, Roossien and Robillard (305) reported the extraction of II^{Mtl} as a dimer from the membrane. From stationary-phase cells, fragments immunologically related to the intact II^{Mtl} were isolated. Limited proteolysis of the purified enzyme with trypsin or chymotrypsin resulted in similar fragments (M_r, 28,000 to 29,000) and inactivated the phospho-HPr-dependent phosphorylation of mannitol. Treatment of spheroplasts (right side out) and inverted vesicles (inside out) with proteases, membrane-impermeable reagents, or polyclonal antibodies raised against purified II^{Mtl} allowed the determination of the intramembrane topology of the enzyme. By following the effects of such treatments on vectorial exchange phosphorylation in which neither phospho-HPr nor its binding site on II^{Mtl} is involved, one can test the effect on the substrate and product binding sites. If, however, the effect of these agents is tested on transport- and phospho-HPr-dependent phosphorylation, the two domains involved in translocation and phosphorylation can be examined too. The results of such studies (145) suggest that II^{Mtl} spans the membrane in an asymmetric orientation. Only a small part of the protein seems to be exposed at the side of the membrane oriented toward the medium. This part could carry the binding site for extracellular mannitol or constitute the opening of a transmembrane channel leading to such a binding site. The first 336 NH_2-terminal amino acid residues have a hydropathy value of +0.90 (187), characteristic of hydrophobic proteins. At least seven regions of this part of the molecule could span the membrane. Interspersed are hydrophilic stretches such that a transmembrane pore could be formed. The last 301 amino acids (hydropathy value, −0.32) are predominantly hydrophilic. This part resembles a globular protein, a large part of which is exposed at the

TABLE 2. Enzymes II of the PTS in enteric bacteria

Enzyme II	Genetic symbol[a]	Map position[a]	Substrates[b]	References
II^{Nag}	*nagE*	16 (15?)	*N*-acetylglucosamine, streptozotocine	156, 196, 392
II^{Glc}	*ptsG* (*cat glcA gpt umg tgl*)	24 (26)	Glucose, methyl α-glucoside, 5-thioglucose, glucosamine, L-sorbose, mannose, 2-deoxyglucose	65, 354, 363
II^{Tre}	*tre*	26 (37?)	Trehalose	221
II^{Man}	*ptsM* (*dgsA manA mpt ptsX*)	40	Mannose, glucose, *N*-acetylglucosamine, 2-deoxyglucose, glucosamine, fructose, methyl α-glucoside	65, 156, 301, 363
II^{Gat}	*gatA*	46	Galactitol, glucitol, 2-deoxygalactitol, arabinitol	72, 192, 197, 284
II^{Fru}	*fruA* (*ptsF*)	47	Fructose, L-sorbose, glucose, mannose, xylitol	100, 196, 354
II^{Gut}	*gutA* (*srlA*)	58 (59?)	Glucitol, 2-deoxyarabinohexitol, galactitol, mannitol, arabinitol, fructose, glucose	192, 197, 339
II^{Mtl}	*mtlA*	81 (79)	Mannitol, glucitol, arabinitol, 2-deoxyarabinohexitol	192, 197
II^{Bgl}	*bglC*	83	β-Glucosides, glucose, methyl α-glucoside, cellobiose	103, 341
II^{Sor}	*sorA*	91	L-sorbose	163, 360, 398
II^{Scr}	*scrA*		Sucrose, glucose	6, 200, 343
II^{Dha}			Dihydroxyacetone	152
II^{Lac}			Lactose	127

[a] The genetic symbols and the map positions on the chromosome of *E. coli* and *Salmonella typhimurium* (values in parentheses) are from Bachmann (18), Lin (208), and Sanderson and Roth (338), respectively. Alternative genetic symbols are included in parentheses.

[b] The underlined compounds exhibit the highest affinity for the corresponding enzyme II. They are most probably its "natural" substrate and inducer. The other compounds are listed according to decreasing affinities.

cytoplasmic side of the inner membrane. It is tempting to speculate that this part or a shorter hydrophilic portion (amino acids 190 to 260) in the amino-terminal part, or both, interact with phospho-HPr to catalyze the phosphoryl exchange reaction.

The membrane-bound II^{Glc}, which forms a complex with III^{Glc} and is coded for by the gene *ptsG* (*glcA*), has also been purified to homogeneity from *Salmonella typhimurium*. Its apparent MW on sodium dodecyl sulfate gels is 40,000, whereas it sediments in the solubilized form with an apparent MW of 105,000 (99). The enzyme is sensitive toward antibodies raised against purified II^{Glc}, and these antibodies inactivate membrane-bound native activity. Again, a characteristic degradation product is formed during purification (M_r, 35,000). Whether transport and vectorial phosphorylation are catalyzed by purified and reconstituted II^{Glc}-III^{Glc} alone or whether additional and as yet unidentified proteins are necessary remains to be shown. In the presence of PEP, enzyme I, HPr, and III^{Glc}, a membrane-bound protein (M_r, 48,000) is labeled in *ptsG*$^+$ wild-type strains which is absent from *ptsG* mutants. The lability of its phospho bond suggests that it might be an N1-phospho-His bond or an acylphosphate group (264).

On the basis of early biochemical experiments, Kundig and Roseman (184) have claimed that the mannose-PTS consists of four proteins. According to these data, extraction of membranes from *E. coli* cells pregrown on glucose medium, with urea, *n*-butanol, and sodium deoxycholate, as well as further purification of the supernatant fraction in aqueous buffer by isoelectric focusing resulted in a hydrophobic protein II-B (M_r, 36,000) and three distinct II-A proteins. Reconstitution of active complexes required, besides phospholipids, the II-B and one of the II-A proteins. Depending on the II-A protein used, phosphorylation activity was specific for fructose, methyl α-glucoside, or *N*-acetylmannosamine, respectively. More recent data on the different enzymes II involved in hexose transport are not easy to reconcile with these data. A more likely interpretation is the presence in enteric bacteria of a mannose-PTS made up of a II^{Man}-III^{Man} complex coded for by the gene locus *ptsM* (consisting possibly of more than one gene; see below), which has a broad substrate specificity and is expressed in a constitutive way. In addition, there exist a glucose-PTS (II^{Glc}-III^{Glc}), a fructose-PTS (II^{Fru}-III^{Fru}), and finally an *N*-acetylglucosamine-PTS (II^{Nag}).

None of the other enzymes II of enteric bacteria has been purified to homogeneity. The question of how many different polypeptides are involved in their activity cannot be answered at present. Only a membrane-bound β-glucoside-PTS (II^{Bgl}) activity has been partially purified. No indication for the existence of a soluble III^{Bgl} was found (306).

In *S. aureus*, a membrane-bound II^{Lac} (M_r, 55,000) has been purified (340) and its interaction with the corresponding III^{Lac} has been characterized (357). Antibodies against the purified enzyme inactivate in crude membrane fractions the lactose-phosphorylating activity. Amino acid analysis of the purified enzyme reveals a low percentage of polar amino acids, a property characteristic of integral membrane proteins. A 35,000-MW protein band reported earlier by the same group to be II^{Lac} (176) is now shown not to be involved in the lactose-PTS of this bacterium but to be an impurity of the partially purified II^{Lac} preparation. From a strain carrying a mutation in III^{Lac}, a defective III^{Lac} was isolated in which a glycine at position 18 is changed to a glutamate. The mutated III^{Lac} could still be phosphorylated by phospho-HPr. Its hydrophobicity increased on phosphorylation and caused it to bind to detergent micelles in a similar manner to that of wild-type III^{Lac}. Nevertheless, the mutated III^{Lac} was unable to catalyze the transfer of its phosphoryl group to lactose, indicating that its interaction with II^{Lac} is disturbed. This interaction thus seems to require an intact NH_2-terminal end of the III^{Lac} (357).

From *Streptococcus faecalis* cells, finally, a glucose-specific membrane-bound II^{Glc} was solubilized by means of the nonionic detergent Aminoxid WS 35 and partially purified. It is unknown whether a III^{Glc} is required in the reaction catalyzed by II^{Glc}, which accepts glucose, 2-deoxyglucose, 5-thioglucose, and mannose (143). Many other enzymes II exist, but the biochemical analysis was restricted to the identification of a membrane-bound phosphotransferase ac-

tivity which was dependent on the presence of enzyme I and HPr.

FUNCTIONS OF THE PTS IN TRANSPORT AND PHOSPHORYLATION

Transport systems are vectorial systems which catalyze the translocation of substrates through membranes. The activity can be measured either in whole cells or, in the case of the purified components, after reconstitution in liposomes. The PTS is unique among the bacterial solute transport systems in that its enzymatic activity can be measured in cell extracts. Incubation of a PTS carbohydrate with PEP and the soluble and membrane-bound proteins results in the formation of a carbohydrate phosphate which can be separated from the substrate and determined quantitatively (198, 387). These simple and reproducible tests have helped greatly in the characterization and purification of the various components and in detailed kinetic studies. The partial reactions of the PTS can also be studied by using the phosphorylated and nonphosphorylated proteins. Furthermore, proteins from various gram-positive and gram-negative bacteria can be tested in homologous and heterologous complementation tests.

Convenient as the in vitro phosphorylation assay with soluble and membrane fractions or purified components of the PTS is, one should be aware that such test conditions are quite different from those in the cells. In general, the concentration of the proteins is low in cell extracts, often below 1% of the values present in vivo. A second disadvantage of the in vitro system is that after sonication or the use of a French pressure cell to break cells, the orientation of the membrane inverts. In such membrane preparations the added PTS proteins and PEP are located on the same side of the membrane as in intact cells, but the substrate clearly is on the wrong side. If the substrate has to first cross the membrane to reach the substrate-binding site of the enzyme II at the inside of such inverted vesicles, the apparent K_m values deduced from the in vitro tests could differ from data obtained in vivo. In the few cases in which such data are available from purified components and from strains having only one enzyme II for a given substrate, the values are similar (145, 148, 192, 363). One could argue that in these cell-free systems only the activity of leaky or nonsealed vesicles is measured (299).

A reasonable alternative is the system developed by Gachelin (108, 109), in which cells are made permeable to small molecules such as PEP, carbohydrates, and carbohydrate phosphates by treatment with low concentrations of toluene. This treatment prevents the leakage of enzymes, preserving the high intracellular concentration of the PTS components.

In contrast to in vitro studies, transport studies with intact cells are hampered by the fact that the carbohydrate phosphates formed during translocation are subsequently metabolized. From this metabolism may originate metabolic intermediates such as pyruvate, lactate, acetate, butanediol, CO_2, and others that can leak out of the cells. Uptake rates estimated from intracellular accumulation of radioactively labeled substrates can be severely underestimated in this way (277). Neither the chemical determination of the intracellular solutes nor measurement of the disappearance of the label from the medium is a solution unless all intermediates are recovered quantitatively. The use of nonmetabolizable analogs or mutants with defects in subsequent metabolism of the phosphate ester is also only a partial solution. In this case the limited capacity of the intracellular compartment and feedback inhibitions of the enzyme II activity limit the accumulation of solutes and complicate determinations of initial rates by restricting the linear part of the reaction to the first few seconds (158, 281, 363). Preparations of right-side-out vesicles from spheroplasts which are devoid of most of their cytoplasmic proteins can still take up PTS carbohydrates, provided that PEP is supplied intravesicularly (159). Obviously, sufficient enzyme I and HPr remains inside the vesicles during preparation.

With using these various methods, the kinetic parameters of reactions 1 to 4 have been measured for a number of PTS systems. Unfortunately, only a few studies have been reported with intact cells or membrane preparations derived from strains having only one enzyme II for a given substrate. In general, enzymes II have a high apparent affinity (0.3 to 50 μM) for their natural substrate. This substrate invariably is the inducer of the system and is catabolized further by the enzymes whose structural genes are clustered in the same operon as the structural gene for the enzyme II (Table 2). For other substrates, e.g., nonmetabolizable analogs or structurally related molecules, the affinities are frequently much lower.

An important feature of the PTS is that the substrate, at the same time as its translocation through the membrane, is phosphorylated in a PEP-dependent vectorial process, also called group translocation. Initially, no free substrate can be detected in the cells. In addition, membrane preparations containing enzymes II catalyze a vectorial carbohydrate-carbohydrate phosphate transphosphorylation which is independent of PEP and the soluble components of the PTS.

$$[^{14}C]\text{Carbohydrate}_{out} + \text{carbohydrate phosphate}_{in} \overset{\text{enzyme II}}{\rightleftharpoons} [^{14}C]\text{carbohydrate phosphate}_{in} + \text{carbohydrate}_{out}$$

In transphosphorylation (204, 290, 291, 316, 320, 324), the enzyme II has the same tight substrate specificity as in the group translocation process. To drive the process, however, high concentrations of the carbohydrate phosphate are required. Both processes, group translocation and vectorial transphosphorylation, raise several important questions, intimately related to the transport process.

(i) Can the PTS phosphorylate substrates from the inside? Vectorial processes related to the PTS transport function are seemingly asymmetric processes requiring the presence of the soluble PTS components or high concentrations (>10 mM) of the substrate phosphate at the inside of the cytoplasmic membrane and lower concentrations (<100 μM) of the substrate at the opposite side. Kaback (158, 159) was the first to use systematically oriented *E. coli* membrane vesicle preparations which lacked most of the soluble PTS components and were right side out. Alternatively, inverted (inside-out) particles have been used. After right-side-out vesicles were preloaded under nonphosphorylating conditions with [^{14}C]glucose, [^{3}H]glucose and PEP were added at the outside. Under these conditions only [^{3}H]glucose 6-phosphate was found in the vesicles, apparently indicating a preferential phosphorylation of external over internal substrate. The validity of this assumption depends, however, on the assumption that no [^{14}C]glucose 6-phosphate was lost during the incubation with [^{3}H]glucose, e.g., by leakage from the vesicles or by exchange phosphorylation (158, 159). Unex-

pectedly, it was observed in such experiments that addition of PEP, HPr, and enzyme I externally to such vesicles primarily stimulated the formation of substrate phosphate at the outside of the vesicles. This would suggest that the active site of the enzyme II was available to the external surface, either because some vesicles were leaky or had inverted membranes or because protein rearrangements occurred within the membrane such that some enzyme II molecules now faced one side of the membrane and other enzyme II molecules faced the other side.

Recently, such studies have been repeated with membrane vesicles from *Salmonella typhimurium* that had the same orientation as intact cells and were tightly sealed (21). According to these studies, both the II^{Man} and the II^{Glc} system are active when the soluble components of the PTS and PEP are trapped inside the vesicles. If supplied from the outside, however, II^{Man} but not II^{Glc} was able to phosphorylate its substrates. These results, if interpreted correctly, suggest that II^{Glc} is oriented asymmetrically in the membrane, whereas II^{Man} is able to phosphorylate its substrates from both sides. The products of II^{Man} were always found on the same side of the membrane as the soluble PTS proteins. Similar results were obtained with intact cells of *E. coli* treated with lipophilic and hydrophilic sulfhydryl reagents (126). II^{Glc} was found to be asymmetric, and II^{Bgl} was symmetric.

II^{Mtl} seems to span the membrane in an asymmetric orientation, with one substrate binding site exposed to the medium and with the phospho-HPr or the substrate phosphate-binding site, or both, oriented toward the inside (145). This system, which is basically irreversible in nonenergized cells (192), does show a vectorial transphosphorylation in energized cells but also in liposomes reconstituted with II^{Mtl} (204). It has been claimed that in vesicles and intact cells a nonvectorial transphosphorylation was even more prominent than vectorial phosphorylation when carbohydrate and carbohydrate phosphate were present at the external surface of the membranes (329). Provided that the majority of the vesicles used in these experiments were right side out and tightly sealed, an assumption challenged in a recent paper (299), this would indicate two populations of enzyme II molecules: one catalyzing a PEP or substrate phosphate-driven nonvectorial transphosphorylation $substrate_{out}$-$substrate\ phosphate_{in}$, the other catalyzing a nonvectorial transphosphorylation $substrate_{out}$-$substrate\ phosphate_{out}$. The vectorial and vectorial transphosphorylation have also been demonstrated for II^{Glc} (290).

A series of experiments have been performed with intact cells to solve the problem of whether phosphorylation of PTS carbohydrates from within is possible. In most of these experiments, di- or trisaccharides were used, which are taken up via non-PTS transport systems and which contain as one moiety a PTS carbohydrate (e.g., lactose, maltose, sucrose, raffinose, lactulose, galactosyl-mannitol, or galactosides containing various glucose analogs). After their hydrolysis by intracellular hydrolases, free PTS carbohydrates are generated within the cells. In strains or in mutants lacking ATP-dependent kinases, the PTS carbohydrates can only be phosphorylated by the different PTSs. Furthermore, when mutants were used which are unable to grow on the non-PTS carbohydrate moiety of the di- or trisaccharide, it could be shown that growth on the PTS carbohydrate moiety required the presence of an intact PTS (65, 359), including the enzymes II. Different data give evidence for (48, 73, 372) and against (162, 201) phosphorylation of PTS carbohydrates from within. Kelker and Anderson (162) showed that [^{14}C]fructose formed by internal hydrolysis of sucrose was excreted by cells of *K. pneumoniae* when unlabeled fructose was simultaneously added to the medium. Similarly, when cells of *E. coli* K-12 were simultaneously offered [^{3}H]glucitol (25 μM) and galactosyl-[^{14}C]glucitol (100 mM), only [^{3}H]glucitol 6-phosphate and [^{14}C]glucitol could be found in the cells during the first few minutes (201; J. Lengeler, *in* G. Semenza and E. Carafoli [ed.], FEBS Symposium 1976: Biochemistry of membrane transport, p. 34).

In one type of experiment with *S. aureus*, when glucose was generated intracellularly from maltose, glucose 6-phosphate was detected inside the cells (48). An external "glucose trap" (glucose oxidase) was present to convert any glucose that leaked into the medium and thus to prevent its reentry through the PTS. It is unlikely, however, that the low-affinity glucose oxidase could compete effectively with the high-affinity II^{Glc} system.

In recent experiments (372) in which [^{14}C]-labeled glucose analogs were introduced as galactosides into *Streptococcus lactis* cells by means of II^{Lac} and after hydrolysis accumulated in the free form inside the cells, these analogs, substrates of the mannose-PTS, were phosphorylated by this PTS under conditions in which their reentry was prevented by the addition of other substrates of the mannose-PTS to the medium. Although phosphorylation required an intact II^{Man}, exit of the dephosphorylated analogs did not. It is tempting to speculate that this enzyme II, which in many respects resembles the II^{Man} of the family *Enterobacteriaceae*, is also able to phosphorylate its substrate from both sides of the membrane, whereas other enzymes II (e.g. fructose-, β-glucoside- or glucose-PTS) are asymmetric (21, 126).

(ii) Are the enzymes II phosphorylated during vectorial phosphorylation? A major question concerning the nature of the phosphoryl group transfer is whether the mechanism is sequential or "ping-pong" and whether phospho-enzyme II intermediates exist. On the basis of kinetic tests, such phosphorylated intermediates have been predicted for several enzymes II (143, 204, 224, 234, 265, 303, 306). Most kinetic data suggest a ping-pong mechanism, according to which the enzyme II is phosphorylated by means of phospho-HPr or phospho-enzyme III. Alternatively, very high substrate phosphate concentrations might substitute in transphosphorylation reactions. In all these cases the same phosphoryl binding site must be involved (143, 234, 265, 290, 291, 306).

The overall stereochemical course of reactions 1 to 4, leading to the phosphorylation of methyl α-glucoside by means of II^{Glc}-III^{Glc} and the soluble PTS components, has been determined (20). This elegant work started with PEP chiral at the phosphorus and showed that from PEP to methyl α-glucoside 6-phosphate the phosphoryl group must have been transferred in total five times, i.e., at least twice between phospho-enzyme III and methyl α-glucoside phosphate, implying a covalent phospho-enzyme II as an obligatory intermediate. Finally, membrane-bound proteins corresponding to II^{Glc} (264), II^{Nag}, and II^{Mtl} of *Salmonella typhimurium* and *E. coli* (386) have been shown recently to be phosphorylated by [^{32}P]PEP in a phospho-enzyme-I-dependent reaction.

From studies with II^{Glc} and II^{Man} of the enteric bacteria (290, 291) sequential Bi-Bi kinetics were postulated for the vectorial transphosphorylation reaction as tested with *n*-butanol-extracted membranes, indicative of the simultaneous presence of two molecules at the active site of a single enzyme II molecule, which in its active form thus should be

a dimer. Phosphoryl transfer would then take place directly from substrate phosphate to the substrate molecule without the participation of a phospho-enzyme II intermediate while both molecules are present simultaneously at the active site(s) of the enzyme. Vectorial transphosphorylation by a sequential, channel-type mechanism, however, involving direct transfer of the phosphoryl groups from carbohydrate phosphates to carbohydrates is difficult to explain. It is easier to envisage by a ping-pong mechanism, involving a phospho-enzyme II intermediate. In accordance with this hypothesis, other studies report ping-pong kinetics for the II^{Glc} and the II^{Man} phosphotransferase systems (234, 295). The question obviously remains unsolved, although phospho-enzymes II and ping-pong kinetics seem more likely.

(iii) Do enzymes II catalyze facilitated diffusion in the absence of phosphorylation? Tight *pts* mutations isolated in different bacteria are unable to grow on the various PTS carbohydrates if no other active transport systems are available. The same result is also observed if these substrates are used at very high concentrations (>50 mM) and when the cells contain active ATP-dependent kinases or other metabolic enzymes for these carbohydrates (65, 278, 281, 353). This apparently rules out any efficient role of nonphosphorylated enzymes II as transport systems that can catalyze facilitated diffusion. Furthermore, no equilibration between substrate in the medium and intracellular substrate is observed in intact cells, supporting the notion that no efficient facilitated diffusion from the outside toward the inside does occur through the enzymes II in the absence of an intact PTS. Facilitated diffusion of PTS carbohydrates via the appropriate enzyme II can, however, occur in enzyme II mutants. In *Salmonella typhimurium* (273), a mutant has been isolated that takes up glucose via II^{Glc} in the absence of concomitant phosphorylation. The mutation results in an altered II^{Glc}, unable to phosphorylate glucose in a PTS-dependent process and with a drastically lowered substrate affinity (K_m, 10 mM compared with 5 μM in the wild type). The maximal velocity of uptake via the mutated II^{Glc} remains basically unchanged. It has been suggested that II^{Glc} is a closed pore which is opened on phosphorylation (273). The present mutation thus could have resulted in a permanently opened pore.

Other data obtained mostly with cells of wild-type strains indicate that at least some nonmutated enzymes II seem to catalyze facilitated diffusion, although with a low efficiency. (a) The non-PTS carbohydrate galactose can be transported by an enzyme II in the absence of phosphorylation in enteric bacterial strains lacking all active transport systems for this sugar. The enzyme II involved seems to be II^{Man} in *Salmonella typhimurium* (271). Introduction of a *ptsI* mutation into this strain does not impair growth on galactose, whereas mutations in *ptsM* do. On the basis of similar experiments with *E. coli* K-12, II^{Glc} was claimed to be involved in this bacterium (172). Since further metabolism of galactose required phosphorylation via galactokinase, free galactose was inferred as the ultimate product of the II^{Glc}-dependent uptake process. (b) When *ptsI* mutants of *E. coli* K-12, synthesizing the lactose permease (*lacY*) and β-galactosidase (*lacZ*) constitutively, were incubated with galactosyl-[^{3}H]mannitol, free [^{3}H]mannitol appeared in the cells (359). After preinduction of II^{Mtl}, mannitol leaked into the medium faster than when II^{Mtl} had not been preinduced, the steady-state level of internal accumulation being reduced to one-fifth. Since the initial rates of uptake were unchanged, the lower level of accumulation was attributed to an enhanced efflux rate. Similar results were obtained with galactosyl-[^{14}C]glucitol when the accumulation of free [^{14}C]glucitol in the *ptsI* strains expressing II^{Gut} constitutively was compared with that in a *ptsI* strain lacking II^{Gut} (J. Lengeler, *in* G. Semenza and E. Carafoli [ed.], FEBS Symposium 1976: Biochemistry of membrane transport, p. 34). Finally, induction of the *mtl* or the *gut* operons from the outside is only possible in tight *pts* mutants if an active enzyme II specific for the inducer is present (see below) (201, 358). (c) The predominant physiological role of bacterial transport systems is promotion of the translocation of the solutes from the medium through the membrane into the cytoplasm. Under physiological conditions and with the metabolic enzymes fully induced, the PTS is virtually irreversible and an asymmetrically operating enzyme II seems appropriate. Irreversible transport systems, however, have one major disadvantage: when toxic analogs are present in the medium or when phosphorylated intermediates accumulate in mutants, the cell needs a detoxification mechanism (15). The intermediates must be expelled either directly in the phosphorylated form or after dephosphorylation by phosphohydrolases. Such an expulsion of free methyl α-glucoside has been observed long ago (109, 123–125, 137–139, 396, 397). After cells had been preloaded with this analog, subsequent addition of an energy source caused an almost complete and rapid displacement of the methyl α-glucoside phosphate from the cells. The material was recovered quantitatively in the medium in the free, nonphosphorylated form. This chasing by energy sources not structurally related to the substrates of the II^{Glc} system is clearly different from the vectorial transphosphorylation discussed above, and must involve intracellular acid phosphatases (94, 125). Such a phosphohydrolase (pH optimum, 6.0) which is rather specific for hexose phosphates is found in *E. coli* K-12 cells. The enzyme is repressed by growth in the presence of glucose, is sensitive to fluoride but not to inorganic phosphate, and is activated by high internal concentrations of carbohydrate phosphates (R. J. Mayer, Ph.D. thesis, University of Regensburg, Regensburg, Federal Republic of Germany, 1980).

It is unclear at present whether efflux is via an enzyme II specific for the carbohydrate that is to be expelled or through a non-PTS transport system. When extrusion of a PTS carbohydrate results from the addition of another PTS carbohydrate, the latter requires a fully active PTS to be able to displace the former substrate. Extrusion is not restricted to substrates of II^{Glc}, but is also observed for substrates of the II^{Man} and to a lesser degree for the enzymes II specific for the hexitols.

Reizer and Panos (288) and subsequently other groups (286, 370, 373) described in gram-positive bacteria a similar process, which they called inducer expulsion. This process also depends on the phosphorylation of the displacing substrate, which need not be structurally related to the substrate phosphate to be displaced. The process involves a step sensitive to fluoride and arsenate, requires a relatively high internal concentration of the displacing substrate, and thus is remarkably similar to the process observed in *E. coli* and other enteric bacteria. An acid hexose 6-phosphate:phosphohydrolase most likely involved in the process has been isolated from *Streptococcus lactis* (371). When 2-deoxyglucose was added to cultures of this bacterium growing on sucrose or lactose, 2-deoxyglucose 6-phosphate accumulated rapidly, the intracellular concentration reaching 100 mM at the steady-state level. The addition of glucose caused expulsion of 2-deoxyglucose after the hydrolysis of the carbohydrate phosphate by the hydrolase mentioned.

Because of a continued PEP-dependent synthesis of 2-deoxyglucose 6-phosphate and its continued dephosphorylation coupled to an efflux from the cells, the overall process promotes a futile cycle in which PEP is dissipated and which, as predicted by Andrews and Lin (15), does serve as an effective detoxification mechanism.

In *Streptococcus pyogenes*, thiomethyl-β-galactopyranoside (TMG) could be expelled by glucose from the cells after its uptake through II^{Lac} and the subsequent dephosphorylation of TMG phosphate by a phosphohydrolase (286, 289). Expulsion was not due to a vectorial transphosphorylation between the incoming glucose and intracellular TMG 6-phosphate, since more of the β-galactoside was displaced than glucose was taken up. Expulsion was inhibited competitively by substrates of II^{Lac} and occurred only after an ATP-dependent dephosphorylation of the TMG 6-phosphate preceded the expulsion. Subsequently it could be shown that the ATP-requiring step was the phosphorylation of HPr at a single seryl residue. Phosphoseryl-HPr is apparently involved in the activation of a phosphohydrolase (78, 79, 285). Further aspects of inducer expulsion related to the regulation of carbohydrate uptake and diauxic growth are discussed below.

A complication in the interpretation of such data obtained with intact cells is that in the streptococci an anion antiport system seems to exist which catalyzes an exchange between inorganic phosphate and hexose phosphates in a process independent of additional energy (220). This exchange bears striking resemblance to inducer expulsion via the II^{Man} system. Its physiological role might be a phosphate-driven uptake of carbohydrate phosphates in the presence of high internal phosphate concentrations (resting cells) or uptake of inorganic phosphate from the medium at the expense of carbohydrate phosphates with concomitant detoxification. A rapid but apparently different exchange process between phosphate and PTS carbohydrate phosphates is also observed in *E. coli* K-12 cells (308). How all these processes are related to the PTS enzymes II, if at all, remains to be shown.

Our knowledge about the different processes catalyzed by the enzymes II in living cells under physiological conditions is still very sketchy. It probably reflects the extreme complexity of the system and the differences between the various enzymes II in different bacteria. A reasonable working hypothesis concerning their role as a group translocation transport system is that enzymes II are pores which can be opened and closed by phosphorylation-dephosphorylation and which are located asymmetrically in the membrane. A high-affinity binding site for the substrates is accessible only from the outside, when the enzyme is phosphorylated via the soluble components of the PTS at the expense of PEP or via the specific carbohydrate phosphates. Binding of a substrate at this stage triggers a conformational switch in the enzyme II and initiates translocation of the substrate through the membrane and subsequent transfer of the phosphoryl group from phospho-enzyme II to the substrate. This in turn reduces the affinity and causes the release of the carbohydrate phosphate into the cell, where the intermediate is normally trapped by subsequent metabolic enzymes. Rephosphorylation of the enzyme II would then restart a new cycle. Facilitated diffusion of the substrates through nonphosphorylated enzymes II (perhaps preferentially from inside to outside) seems to be involved in inducer expulsion or in the secretion of PTS carbohydrates generated inside the cells by hydrolysis of oligosaccharides when their efficient metabolism is not possible.

INVOLVEMENT OF THE PTS IN CHEMOTAXIS

Bacteria sense chemotactic stimuli by means of a series of chemoreceptors, which are multifunctional, membrane-bound protein complexes. Binding to or dissociation of a substrate from its chemoreceptor constitutes the stimulus which eventually triggers a positive or negative behavioral response. Transport or metabolism of the stimulating substrate is not a prerequisite for chemotaxis. The response of a bacterium to a stimulus is transient. A rapid excitation alters the frequency of the run-tumble periods such as to bias the normal random walk to produce a net movement in the favorable direction. Run-tumble episodes reflect counterclockwise and clockwise rotation of the flagellar rotary motor, which during excitation must somehow react to temporal changes in concentrations at the chemoreceptor. The process involves a series of conformational changes at the reception site (chemosensor), at the flagellar rotary motor (tumble regulator), and diffusible molecules. The complexity of the system, which integrates the signals from all chemoreceptors, is reflected in the large number of *fla*, *mot*, *che*, and chemosensor genes present in enteric bacteria. At the same time, stimulation of a chemoreceptor triggers a slower process, adaptation, by which the original nonbiased pattern of flagellar rotation is reestablished. Numerous aspects of bacterial chemotaxis have been reviewed recently (42, 134, 177).

Most chemotactic responses in the enteric bacteria are mediated by one of four different methyl-accepting chemotaxis proteins (MCPs). A different class are the enzymes II (2, 191, 197, 255). Stimulation through these enzymes II eventually triggers the same response as MCP-mediated excitation, although the pathway seems to differ substantially and perhaps completely.

Thus for all enzymes II, the processes of transport-phosphorylation and chemoreception are tightly coupled (2, 3, 197, 231). Cells lacking a functional enzyme II (or enzyme III) were unable to transport and phosphorylate substrates of that particular system and to react chemotactically to these substrates while reacting normally to other PTS carbohydrates. A series of mutants has been selected which are chemotactically negative for a single PTS carbohydrate. Invariably the corresponding enzyme II was lacking or had an altered activity. These included mutations in II^{Mtl} (191, 197, 203), a system known to consist of only one polypeptide. Despite an intensive search involving positive selection procedures, no mutant has been found that still has an enzyme II-dependent transport system but lacks a chemotactic response. Furthermore, substrate specificity and affinity measured by transport-phosphorylation tests and by chemotaxis assays were similar. The high affinity of substrates as measured in transport tests was reflected in low threshold values in chemotaxis. This correlation is even found in mutants which, owing to a mutation in *mtlA* or *gutA*, have lowered affinities of II^{Mtl} and II^{Gut} for their substrates (197). Such a strict similarity is expected if transport-phosphorylation and chemoreception are catalyzed by the same transmembrane enzyme II, especially if the system consists of only one polypeptide, as for II^{Mtl}.

Mutants chemotactically inactive toward all PTS carbohydrates have been isolated. These react normally to attractants or repellents signalling through one of the four known MCPs. They invariably were mutated in the general PTS proteins enzyme I or HPr or both (2, 191, 197, 231). In contrast, mutants with defects in the MCP-dependent signal

transduction responded normally to enzyme II-mediated stimuli (134, 255, 263, 362). From such results we have to conclude that excitation by PTS carbohydrates does not involve the initial part of the MCP-dependent signal transduction pathway.

From the behavioral response of a series of mutants with mutations in the different PTS proteins, it has been concluded that for enzyme II-mediated chemotaxis the decisive stimulus is the reversible alteration of these proteins during the uptake of a substrate between the phosphorylated and dephosphorylated forms (197). The signal, whose exact nature is still unknown, may involve the physical contact between an enzyme II and another chemotaxis protein or a direct sensing of enzyme II alterations. Alternatively, signalling may occur via a biochemical linkage, e.g., through perturbation of a pool of phosphorylated compounds related to the PTS (263, 360). In this connection it is interesting to note that the coupling between the MCP signal transducers and the flagellar rotary motor also appears to be indirect, probably involving a diffusible chemical. This molecule seems to be related structurally to phosphorylated nucleotides. A candidate is cyclic guanosine monophosphate or a closely related compound whose synthesis depends on ATP (16, 32, 110, 134, 350).

A role for adenylate cyclase has been implicated in the signal transduction emanating from enzymes II (33). The activity of adenylate cyclase is regulated via III^{Glc} (see below), and the enzyme is involved either directly in the synthesis of cyclic guanosine monophosphate (33, 349, 401) or indirectly by regulating the activity of a guanylate cyclase (215, 368, 375). The existence of *cya* mutants which lack any detectable intracellular cyclic adenosine monophosphate (cAMP) but react normally in PTS chemotaxis eliminates this nucleotide as a direct signal transducer molecule, whereas a role of cyclic guanosine monophosphate remains to be proven (B. Taylor, personal communication; A. Vogler, Ph.D. Thesis, University of Regensburg, Regensburg, Federal Republic of Germany, 1984).

Final proof for a central role of the PTS in enzyme II-mediated chemotaxis is that integration of the corresponding stimuli or signals and the adaptation to such signals also involves the PTS proteins. Several PTS carbohydrates, taken up via different enzymes II, complete with each other (3, 191). By using non-PTS compounds, such as glucose 6-phosphate, which inhibit enzymes II at the inside but which themselves do not act as attractants in chemotaxis assays, it could be demonstrated that the competition is at the level of the PTS (263).

In enteric bacteria, the MCP chemosensors are highly evolved molecules specifically adapted to their function in chemoreception and signal transduction-adaptation. The four MCPs, in combination with a few periplasmic binding proteins of certain transport systems, recognize the majority of attractants and repellents. This broad specificity contrasts with the enzymes II, all of which are active in chemoreception (197). They are, however, not chemoreceptors in the conventional sense, i.e., molecules specifically adapted to chemoreception. Rather, the enzymes II are both involved in transport and metabolism and also in chemotaxis. They trigger a signal transduction pathway that is partly or completely different from the MCP-dependent transduction pathway.

GENETICS OF THE PTS

Apart from the biochemical characterization of the constituents of the PTS, the study of their multiple functions in transport, regulation, and chemotaxis has benefitted most from the isolation of mutants defective in one or more components of the PTS and the mapping of their respective mutations. As early as 1949 (17, 87, 365), such mutants had been isolated inadvertently as strains with a pleiotropic defect in the fermentation of numerous carbohydrates. Furthermore, mutants had been isolated with specific defects in the uptake and phosphorylation of glucose and methyl α-glucoside in *E. coli* K-12 or *Salmonella typhimurium* and of mannitol in *S. aureus* and *K. pneumoniae* (90, 123, 245). From the analysis of such mutants came the first unambiguous evidence for a direct role of the PTS in uptake, phosphorylation, and metabolism of the PTS carbohydrates. The isolation of specific mutations that suppressed the pleiotropic defects due to a mutated PTS also gave the first evidence for an indirect involvement of the PTS in the metabolism of non-PTS carbohydrates such as lactose, maltose, melibiose, or glycerol (17, 24, 241, 262, 382). The names given to the genetic loci involved were as variable as the ideas on the nature of the different mutations and the processes they affected (Table 2). To avoid further divergence in the genetic nomenclature of genes coding for the different PTS functions, Lin (208) proposed a unifying nomenclature. In accordance with the proposal of Demerec et al. (74), it requests that all genes belonging to an operon or a regulon should be designated by the same three-letter code and that different operons or regulons should be given a different code. Consequently, the structural genes coding for the general PTS proteins HPr and enzyme I were designated *ptsH* and *ptsI*, respectively, since together with the promoter sequence *ptsHp*, they form the *pts* operon. Lin suggested, furthermore, that the genes for the substrate-specific enzymes II which are organized in an operon or a regulon together with the structural genes for the corresponding metabolic enzymes should be designated according to these. This nomenclature is now generally accepted for the enteric bacteria (18, 338), with a few exceptions (see below), for *S. aureus*, *B. subtilis*, and the streptococci. It takes into consideration the fact that the structural genes for the enzymes II are not a part of a *pts* operon or regulon, but rather form units with the corresponding metabolic enzymes. This view is strongly supported by the fact that a repressor-activator specific for the major metabolic substrate regulates most of these operons (Table 2).

Unfortunately, another proposal for a uniform nomenclature of the PTS has been suggested which emphasizes a biochemical rather than a genetic point of view (59). It does not comply with the accepted rules of genetic nomenclature and has led to considerable confusion; we discourage its use. A second cause of confusion in the genetic nomenclature of the PTS is the fact that mutations within the same genetic locus but with different phenotypes have been given a new genetic symbol instead of an allelic number before the existence of a new gene had been established unequivocably (see, e.g., the symbols *cat*, *umg*, *gpt*, *tgl*, and *ptsG*, or *iex*, *gsr*, *tgs*, and *crr* in Table 2).

In this review we will use the nomenclature system of Lin (208; Table 2). Included are the known genes for fructose metabolism which constitute a *fru* operon (regulon): *fruA* (formerly *ptsF*) for II^{Fru}, *fruB* for III^{Fru}, and *fruK* for fructose 1-phosphate kinase, together with a regulatory gene *fruR* which maps close to the *leu* gene locus. Exempt from this nomenclature system are the symbols *ptsG* for II^{Glc} and *ptsM* for II^{Man} (which correspond to *glcA* and *manA* in the Lin nomenclature system) for the following reasons. (i) As far as is known, the structural genes *ptsG* and *crr* do not

form an operon or a regulon with each other or with the remaining glucose degradation enzymes also involved in glycolysis, and III^{Glc}, the product of the gene *crr*, has a general role in carbohydrate transport and metabolism. (ii) The gene locus *ptsM* does not form an operon together with *manI*, the structural gene for the enzyme mannose 6-phosphate isomerase, also involved in cell wall synthesis. (iii) Both symbols have been used extensively throughout the biochemical literature.

Mutant selection procedures

Numerous methods are available to select for mutants with defects in the general PTS proteins or in the substrate-specific enzymes II and their regulatory genes. They rely, however, on a few general principles which are discussed here.

(i) Owing to their relatively broad substrate specificity, most enzymes II take up and phosphorylate nonmetabolizable substrate analogs, which accumulate as toxic phosphate esters. Most enzymes II are inducible, but not by the toxic analogs. Consequently, cells preinduced for an enzyme II or mutants expressing the enzyme II constitutively are sensitive, whereas among the survivors, mutants synthesizing an inducible or inactive enzyme II can be found (22, 65, 72, 101, 191, 232, 284). Among the analog-resistant strains are also found *ptsI* mutants (PTS-negative phenotype), *ptsH* mutants (Fru^+, PTS negative), or mutants with defects in the *cya* or *crp* genes. Mutants containing tight *ptsI* or *cya-crp* mutations are negative for most carbohydrates owing to lowered intracellular levels of cAMP and consequent failure to express the corresponding genes.

Streptozotocin is an interesting analog of *N*-acetylglucosamine and is probably the most potent antibiotic known at present for use against gram-positive and gram-negative bacteria containing a functional *N*-acetylglucosamine-PTS. The drug [2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranoside] is taken up in enteric bacteria via II^{Nag} (12, 194–196). Streptozotocin 6-phosphate cannot be metabolized and generates the highly toxic and mutagenic diazomethane that kills the cells. Mutants defective in any PTS transport system or metabolic pathway can be isolated (194, 196, 343, 360).

(ii) Drugs and antibiotics taken up through non-PTS transport systems may also be used to select for mutants with defects in the PTS functions. PTS-negative mutants have in general lowered cAMP levels, take up inducer at a lower rate, and grow more slowly (see below). Consequently, they are more resistant to antibiotics (e.g., nalidixic acid or amino glycosidic drugs) which act only on fast-growing cells and are fully resistant to drugs taken up via inducible transport systems, for instance fosfomycin (9, 61, 232). Any drug taken up via an inducible transport system and any phage or bacteriocin whose receptor requires cAMP for its synthesis can obviously be used in this type of selection, e.g., phage λ in $lamB^+$ strains carrying a functional lambda receptor (31, 43).

(iii) Mutants with a defect in the catabolism of a PTS carbohydrate frequently accumulate phosphorylated and toxic intermediates during growth in the presence of this PTS carbohydrate. Secondary mutants with defects in the corresponding enzyme(s) II or PTS-negative strains are found among the survivors (22, 64, 65, 100, 191, 196, 358, 372). In *E. coli* K-12, several naturally occurring carbohydrates are taken up through an enzyme II and phosphorylated but not metabolized further, such that the wild-type strain resembles a sensitive mutant. These are D-arabinitol, xylitol, and L-sorbose, taken up through II^{Mtl}, II^{Fru}, and II^{Glc}, respectively (192, 284, 354, 360).

(iv) From strains with defects in the structural gene of an enzyme II, suppressor mutations can be isolated which allow growth on substrates of the missing enzyme II by a constitutive expression of a closely related enzyme II (72, 155, 199, 201, 339).

It must be reemphasized that the phenotype of the different mutants varies strongly from one organism to another. Thus one must choose between the various selection procedures, depending on whether more than one enzyme II for a carbohydrate is available, whether non-PTS transport systems are present, and whether the enzymes II are inducible or constitutive.

The *pts* operon

Mutations affecting the expression of enzyme I or HPr in *E. coli*, *Salmonella typhimurium*, and *K. pneumoniae* have been mapped and found to cluster in the *pts* operon (60–62, 96, 114, 311, 312, 381). The published gene order given for *E. coli* K-12 is · · · *cysA* · · · *crr ptsI ptsH ptsHp* · · · *cysK lig supN* · · · . Among the *ptsI* mutations are some that result in a temperature-sensitive enzyme I and provide strong evidence that *ptsI* is the structural gene for this protein (41, 50, 66, 96). Mapping data giving the location of many point mutations relative to a series of deletions which covered part or all of the *pts* operon suggested the existence of a *pts* operon containing a promoter *ptsHp*, the proximal *ptsH*, and the distal gene *ptsI* (35, 115, 312).

A detailed fine structure map of the *pts* region of *Salmonella typhimurium* was constructed (59) by mapping a series of point mutations relative to deletions selected after treatment of cells with nitrous acid and the toxic analog 1,2,4-triazole (62) or the antibiotic fosfomycin (61). From these data, the following gene order was deduced: · · · *cysA cysK* · · · *ptsHp ptsH ptsI crr* · · · *supN* · · · . The gene order of *pts* and *crr* genes given for *Salmonella typhimurium* is inverted compared with the order given for *E. coli* K-12. The latter order has been confirmed by several independent groups (45, 96, 311, 312, 381). Recent results with cloned DNA fragments from the chromosome of *Salmonella typhimurium* containing this region have thrown doubt on the published gene order (249). Plasmids containing the *crr* gene and adjacent regions complemented *cysA* and *crr* mutations but did not complement *pts* deletion mutations, nor did they express in the maxicell system polypeptides corresponding to enzyme I and HPr. Additional mapping data with phage P22 and further deletions covering *cysA*, *crr*, and *ptsI* indicate that most probably the gene order of *Salmonella* strain in this region is similar to the one found in *E. coli* K-12. The previous gene order relied primarily on three-point factor crosses in which transductants were selected for one marker but not purified before being tested for the linked markers. Thus the minority class of double recombinants could easily have been masked by surviving parental cells. In *Salmonella typhimurium*, point mutations in *ptsH* have been isolated which prevent the efficient expression of *ptsI* (62), thus resembling polar mutations. Furthermore, a promoter which reduces the expression of *ptsH* and *ptsI* has been located between *cysK* and *ptsH*. These results suggested the existence of a *pts* operon, made up of the promoter proximal gene *ptsH* and distal gene *ptsI*. In the original mutant strains the level of enzyme III^{Glc} was comparable to the level in a wild-type strain. This was taken

as an indication that *crr* is transcribed from its own promoter (60).

Physical mapping of the *pts* operon and the surrounding DNA by using restriction endonucleases and cloning of the various DNA fragments into plasmids or phage λ derivatives allowed expression and identification of the corresponding gene products in the mini- or maxicell system as well as by immunochemical methods (31, 75, 188, 230, 249). According to these data, two proteins are coded for by the genes *ptsH* and *ptsI*: HPr (M_r, 9,500) and enzyme I (M_r, 68,000).

These mapping studies confirmed furthermore that *ptsH* is the promoter proximal gene. After δ-mutagenesis of cloned fragments, the effect of a second, weak promoter located between *ptsH* and *ptsI*, whose activity had been observed previously in certain *ptsH* mutants (115), became visible (45).

The existence in *E. coli* K-12 of a *ptsJ* gene has been proposed, defects in which resemble tight *ptsI* mutations and map close to this gene (31). The existence of such a gene is unlikely, since according to the cloning data no unaccounted-for space exists adjacent to *ptsI*. All *ptsJ* mutants carry the strong polar mutation *gal-3* (4) which causes galactose sensitivity in strains of *E. coli* K-12 and could be responsible for the inability of *ptsJ* mutants to grow on lactose or melibiose at 42°C. Thus *ptsJ* is a new allele of *ptsI* rather than a new gene of the *pts* operon.

It is generally agreed by now that the genes *ptsH* and *ptsI* form the *pts* operon together with a major promoter *ptsHp* located in front of *ptsH* and perhaps a weaker promoter *ptsIp* located between the two structural genes. Both are expressed in a constitutive way. Similarly, there was general agreement that the structural gene *crr* is tightly linked to the gene *ptsI* but not regulated by the same promoter as the *pts* operon. *pts* mutants of *Salmonella typhimurium* containing either point mutations or deletions still expressed normal levels of enzyme III^{Glc} (61, 62, 346, 347). Furthermore, it was generally agreed that when *pts*-negative mutants lacked III^{Glc}, this was due to secondary mutations. Final proof for this assumption seemed to be the cloning of DNA fragments from *Salmonella typhimurium* (249) or *E. coli* (44–46, 230) lacking the promoter-proximal part of the *pts* operon but still expressing *crr* and synthesizing III^{Glc}. The *crr* DNA fragments from *E. coli*, cloned together with a truncated *ptsI* fragment, resulted in normal expression of III^{Glc}. In *Salmonella typhimurium*, however, the only plasmids giving large amounts of III^{Glc} had the *crr* gene fused to a secondary *bla* promoter of the vector, whereas a low level of expression of *crr* from its own promoter was observed. The *crr* gene might be autoregulated by III^{Glc} (249).

Recently, De Reuse et al. (75, 75a) cloned a DNA fragment carrying the three genes *ptsH*, *ptsI*, and *crr* from *E. coli*. The products coded for by the genes on this fragment and on a series of subclones were identified in complementation tests and in the maxicell system after fusion to the thermoinducible p_R promoter of bacteriophage λ. These were HPr (M_r, 9,000), enzyme I (M_r, 68,000), and III^{Glc} (M_r, 21,000 and 22,000). The genes *ptsI* and *crr* are separated by a maximum of 200 base pairs (bp) and transcribed in the same direction. When the carboxy-terminal part of *ptsI* was fused to the p_R promoter of bacteriophage λ, the gene *crr* came under its control, indicating that no strong functional transcription termination signal is located between *ptsI* and *crr*. Contrary to results discussed above (44, 46, 61, 230, 249), deletion of the *ptsHp* DNA fragment or inversion of *crr* fragments suggests that *crr* is expressed together with *ptsH* and *ptsI* from the promoter *ptsHp* and thus would be part of the *pts* operon. Plasmids expressing *crr* from its physiological promoter complement *crr* mutations in vivo. These plasmids produce enzyme I in the maxicell system, but surprisingly no enzyme III is detected. This protein is only found after removal of *ptsH* or *ptsI* or both and then expressed probably from an unphysiological promoter located on the cloning vector. This might indicate a strong control of *crr* expression by Enzyme I or HPr or both. From sequencing data of the DNA (416 bp) immediately preceding *ptsH*, De Reuse et al. (75a) were able to detect a classical consensus promoter sequence, an open reading frame (starting with an AUG at +163, and ending with a single UAA stop codon) of 255 bp (85 amino acids; M_r, 10,666) preceded by a Shine-Dalgarno consensus sequence. The location of the messenger ribonucleic acid start site was determined by using the classical S1 nuclease mapping technique. Finally, a putative binding site for the cAMP binding protein receptor was observed in the −35 region.

The genes *ptsH* and *ptsI* are separated by a 44-bp intercistronic region (between the UAA stop of *ptsH* and an AUG triplet) corresponding to a poor Shine-Dalgarno sequence but not to a promoter sequence. This AUG is followed by an open reading frame whose 17 first amino acids fit with the *Salmonella typhimurium* amino-terminal end of enzyme I (391) except for two serine residues instead of arginine residues in positions 3 and 8. By contrast to the results of Weigel et al. (390), it was found that the amino acid sequence of HPr from *E. coli* and *Salmonella typhimurium* differ in several areas, especially between residues 62 to 71 (75a). In a recent report (282), it was shown that the published primary structure of HPr from *Salmonella typhimurium* (390) was in error. The corrected sequence agrees with the data obtained from DNA sequence analysis of the *E. coli ptsH* gene (75a).

Gene loci for enzymes II and enzymes III

In contrast to *ptsH* and *ptsI* mutants with their pleiotropic, carbohydrate-negative phenotype, mutants defective in a single enzyme II should be negative only for substrates taken up via that particular enzyme II. The negative phenotype is not necessarily observed on growth plates and in fermentation tests, since almost any PTS carbohydrate is taken up via more than one enzyme II (Table 2). To obtain a negative phenotype, the presence of additional mutations is usually required. Thus, *ptsG* and *crr* mutants were among the first to be isolated as strains resistant against methyl α-glucoside (17, 123, 138), or insensitive to the glucose effect (97). An understanding of their complex phenotype, however, was possible only much later (65, 171, 278).

Mutations affecting the II^{Glc}-III^{Glc} system. From a strain of *E. coli* K-12 lacking all other transport systems for glucose except II^{Glc}-III^{Glc}, isogenic derivatives with defects in II^{Glc} or III^{Glc} due to mutations in the genes *ptsG* or *crr* have been isolated (196, 197). Both types of mutants were unable to take up or phosphorylate substrates of the glucose-PTS including glucose itself and were resistant against toxic glucose analogs such as methyl α-glucoside, 5-thioglucose, or 2-deoxyglucose. Furthermore, glucose was unable to exclude other carbohydrates or to cause diauxic growth in such mutants. Finally, although *ptsG* mutants grew normally on other carbohydrates, the *crr* mutants were unable to grow on a series of non-PTS carbohydrates (e.g., succinate). This is exactly the phenotype found with a large series of *ptsG* and *crr* mutants selected previously in *Salmonella typhimurium* or *E. coli* K-12 from strains which carried

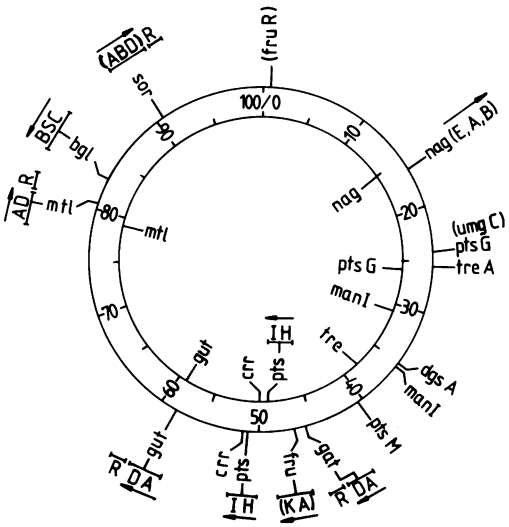

FIG. 2. Simplified genetic map of *E. coli* and *Salmonella typhimurium*, indicating the genes involved in the PTS. The genetic symbols and the map positions on the chromosome of *E. coli* (outer circle) and *Salmonella typhimurium* (inner circle) are from Bachmann (18) and Sanderson and Roth (338), respectively. When the gene order within an operon is not known, the gene symbols are given in parentheses. Arrows above operons indicate the direction of transcription.

certain additional mutations in *ptsI* (resulting in a temperature-sensitive enzyme I or low residual levels of enzyme I) or still contained II^{Man} (65, 173, 174, 248, 260, 327, 328, 344, 347). In *E. coli* K-12, the II^{Glc}-III^{Glc} system is clearly the major glucose transport system. It is sufficient to allow rapid growth on glucose, to ensure high uptake and phosphorylation rates, and to cause exclusion of other carbohydrates. None of the other systems can substitute effectively for it (65, 196, 197).

All mutations affecting the expression or activity of II^{Glc} map at 24 min on the chromosome of *E. coli* K-12 and at 26 min in *Salmonella typhimurium* (Fig. 2). These include the *cat* (218, 374), *gpt* (65), *umg* (154, 156), *tgl* (41, 98), and *ptsG* (*glcA*) (196) mutations described above, as well as the *ptsG* mutations of *Salmonella typhimurium* (231, 232, 273, 275, 278, 333, 363). In *Salmonella typhimurium* (and in contrast to *E. coli*), II^{Man} is as efficient as the glucose-PTS in uptake and phosphorylation of glucose. From both bacterial strains, mutants resistant to high concentrations of 3-fluoro-3-deoxyglucose or 2-deoxyglucose have been isolated (65, 232, 363). Since the analogs enter the cell by the glucose- and the mannose-PTS, *ptsG ptsM* double mutants are to be expected among the survivors. They were indeed found, as indicated by a lowered glucose and 2-deoxyglucose uptake in such mutants. Speculations on the existence of a common subunit or a common regulatory component for both systems to explain the complex phenotype of these mutants seem unnecessary (363). The presence of mutations in *E. coli* in a gene locus *umgC*, which maps close to *ptsG* and causes a constitutive expression of II^{Glc}, further supports the hypothesis that *ptsG* and *umg* designate the same gene locus for II^{Glc} activity. Other mutations that affected the glucose-PTS activity were called *tgl* (98), although their phenotype basically resembles other *ptsG ptsM*$^+$ strains of *E. coli* K-12. Unfortunately, the II^{Man} activity was neither eliminated nor tested for in the *tgl* mutants. Instead, a model was proposed according to which the glucose-PTS is made up of two different subunits, coded for by the genes *ptsG* and *tgl*. The subunit coded for by the *tgl* gene would be necessary to catalyze transport, and the *ptsG*-coded subunit would be necessary to phosphorylate substrates. *tgl* would be a mutation altering a II^{Glc} molecule in such a way that no substrate can reach the active center of the molecule in intact cells while its phosphorylation by phospho-III^{Glc} (as measured in vitro) is still possible. The reverse mutation seems to have been isolated in *Salmonella typhimurium* (273), in a mutant for which II^{Glc} still acts as a substrate-specific pore, but is unable to phosphorylate a substrate during translocation. The mutation maps in *ptsG* and changes the apparent affinity of the mutated II^{Glc} for glucose from 5 μM to 10 mM. Interestingly, the *tgl* mutation altered the affinity of the II^{Glc} too, further supporting the hypothesis that *ptsG* is the structural gene for II^{Glc}. Final proof, however, must await cloning, physical mapping, sequencing of the DNA, and identification of the *ptsG* gene product.

As discussed previously, the *crr* gene has been cloned from *E. coli* K-12 (44, 75, 230), and from *Salmonella typhimurium* (249). The latter gene has been sequenced completely, giving unequivocal proof that it is the structural gene for III^{Glc} (249). A special class of *crr* mutations, called *iex* (44, 175, 260), has been described recently. Since *iex* mutations caused altered behavior in regulation, they are discussed below.

Mutations affecting II^{Man}. Mutants of *E. coli* K-12 which lack II^{Glc}-III^{Glc}, other possible active transport systems for glucose, and glucokinase (coded for by the gene *glk*), are still able to grow slowly on glucose (65). Its transport and phosphorylation in such mutants is via II^{Man}. Originally detected in membranes of *E. coli* K-12 cells, pregrown on glucose, as a PTS able to phosphorylate glucose, fructose, mannose, methyl α-glucoside, and *N*-acetylmannosamine, II^{Man} was considered a glucose-PTS (182). In *E. coli* cells, however, it is a minor system for glucose or methyl α-glucoside but the major transport system for mannose (65, 196).

Mutations in the gene locus *ptsM*, originally called *mpt* by Curtis and Epstein (65), *ptsX* by Jones-Mortimer and Kornberg (153), and *manA* according to the Lin nomenclature system (see reference 196) affect this II^{Man} (Fig. 2). Similar mutations have been isolated in *Salmonella typhimurium* (232, 273, 333). II^{Man} has an unusually broad substrate specificity, even for an enzyme II (Table 2). According to the biochemical data of Kundig and Roseman (184), this is due to the presence of three different II-A proteins, each of which has a different substrate specificity. With the use of a series of mutants lacking II^{Fru}, II^{Glc}, or II^{Nag}, or more than one of the above, independent II^{Man}-negative derivatives have been isolated (65, 100, 153, 196, 301). In each of these selections, the selective pressure was on resistance to one carbohydrate or on the inability to grow on one carbohydrate. Elliott and Arber (92) selected strains with a low plating efficiency for bacteriophage λb221cI26. Such Pel$^-$ mutations also map in the *ptsM* gene locus, and the mutants lack II^{Man} activity. Despite this diversity in the selection methods used, all the mutants isolated thus far were invariably found to lack transport and phosphorylation activity for all the II^{Man} substrates mentioned in Table 2. It seems highly unlikely that only the predicted pleiotropic mutants affected in II-B should have been found but never the single mutants affected in either II-A^{Glc}, II-A^{Man}, or II-A^{Nag}. Thus we are left with two possibilities: either the mutants exist but have never been identified or the original biochemical data were incomplete. The recent identification

(386) of a III^{Man} which together with a membrane-bound II^{Man} gives in reconstitution experiments the full transport spectrum ascribed to the original mannose-PTS (184), supports the notion that more than one membrane-bound enzyme is involved in the phosphorylation of glucose, mannose, and fructose.

Mutations affecting II^{Fru}. In the enteric bacteria, II^{Man} takes up fructose (K_m, ca. 10 mM) and phosphorylates it to fructose 6-phosphate (100, 101, 171). In addition, the enteric bacteria synthesize a fructose-PTS (K_m, 10 μM) inducible by fructose and called II^{Fru}. As shown by Fraenkel for *E. coli* K-12 (104, 105), and by Anderson and coworkers for *K. pneumoniae* (128, 162), the II^{Fru} system yields fructose 1-phosphate. Except for the membrane-bound II^{Fru} coded for by the gene *fruA* (formerly *ptsF*), the system probably comprises a soluble III^{Fru} (386), formerly called K_m factor (379), and possibly an HPr-like protein (also called FPr or pseudo-HPr [59]). All mutations affecting II^{Fru} map at 47 min, closely linked to the gene *fruB* (formerly *fpk*) coding for the enzyme fructose 1-phosphate kinase (11, 153) (Fig. 2). Both activities are coordinately inducible by fructose, probably together with the III^{Fru}, and are regulated by a gene *fruR* (formerly Xtl^s) (284). Mutations in this regulatory gene result in constitutive expression of all activities except FPr and a high sensitivity toward xylitol and L-sorbose (284, 354). In view of these data, it seems safe to conclude that all genes belong to a *fru* operon or regulon and thus should be renamed accordingly.

Mutations affecting II^{Nag}. *N*-Acetylglucosamine can be taken up both via II^{Man} and via II^{Nag}. II^{Nag} is induced by growth on this substrate (196, 392). The structural gene for II^{Nag}, *nagE*, is clustered in a *nag* operon or regulon together with the genes *nagA* (for the enzyme *N*-acetylglucosamine 6-phosphate deacetylase) and *nagB* (for glucosamine 6-phosphate deaminase) (156, 196) (Fig. 2). II^{Nag} also recognizes the nonmetabolizable analogs methyl-α-*N*-acetylglucosamine, iodo-*N*-acetylglucosamine, and the antibiotic streptozotocin, but not glucosamine. This hexosamine normally enters the cells via II^{Man} or via II^{Glc}-III^{Glc}.

Mutations affecting the hexitol-PTS. In enteric bacteria, the three naturally occurring hexitols D-mannitol, D-glucitol (formerly sorbitol), and galactitol (formerly dulcitol) are PTS carbohydrates. The similarity in their metabolic pathways, in the enzymes II and the remaining enzymes involved in hexitol catabolism, but especially in the arrangement of the genes coding for and regulating these enzymes is obvious (22, 161, 191–193, 203, 222, 314, 358, 366). In each case, the structural gene *A* for the enzyme II is promoter proximal and linked to the gene *D* for the corresponding hexitol phosphate dehydrogenase. Adjacent to the gene *D* but outside of the operon is the gene *R* for a repressor, which recognizes the major substrate of the enzyme II as an inducer (72, 155, 201) (Fig. 2). DNA fragments containing the *mtl* (186, 187) and the *gut* (formerly *srl* or *sbl*) operons (37, 64, 227, 393) have been cloned and physically mapped, and the *mtlA* gene has been sequenced (187). In agreement with previous data, it was found that only II^{Mtl} (M_r, 60,000) and the mannitol phosphate dehydrogenase (M_r, 40,000) are coded for by the *mtl* operon. No evidence was found for a cAMP-CAP binding site in front of the *mtlAp* promoter on the sequenced fragment. The *mtl* operon has long been recognized as one whose expression requires only low amounts of cAMP (201). Recently, a new type of mutation has been found to map in the *gut* operon (339). From preliminary mapping data and unpublished biochemical evidence it was concluded that a gene *gutB*, coding for a III^{Gut}, was affected. The published evidence, however, is not yet sufficient to support the hypothesis.

Mutations affecting other enzymes II. Many laboratory strains of *E. coli* K-12, *Salmonella typhimurium*, and *Shigella*, used traditionally in genetic and biochemical experiments, can only use a limited number of carbohydrates for growth, compared with strains isolated freshly from natural environments or strains of the tribe *Klebsielleae*. The genes for such metabolic pathways can be turned off and thus become cryptic, or they are localized on metabolic plasmids which may be present in or absent from the strains. Among those genes there are some coding for PTS-dependent metabolic pathways.

(i) Many strains of the enteric bacteria are unable to metabolize aliphatic or aromatic β-glucosides (Table 2), although these are excellent carbon sources for others (71, 341). Spontaneous Bgl^+ derivates can be isolated. Activation of the genes seems to involve an IS1- or IS5-mediated insertion of DNA into or close to gene *bglR*, apparently the promoter of the *bgl* operon (293). Further genes include *bglC*, the gene coding for II^{Bgl}, *bglB*, the gene coding for a β-glucoside 6-phosphate phosphatase, and *bglS* (Fig. 2). According to Prasad and Schaefler (283, 341), the latter gene codes for a positive regulatory element. It could, however, also code for a transport protein in the absence of which the system cannot be induced. Partial purification of the β-glucoside-PTS, on the other hand, gave no indication of the presence of a soluble III^{Bgl} (306).

(ii) In some strains of the enteric bacteria, the genes responsible for the uptake and fermentation of sucrose are located on metabolic plasmids (343), whereas in other strains and in the klebsiellae these are found on the chromosome (6,200). Cloning, physical mapping, and the expression of the plasmid-coded sucrose genes in the maxicell system showed that the genes are clustered in a *scr* operon (R. Ebner, M.D. Thesis, University of Regensburg, Regensburg, Federal Republic of Germany, 1983). These include *scrA*, the gene for II^{Scr} (M_r, 60,000), *scrB*, the gene for sucrose 6-phosphate hydrolase (M_r, 55,000), and *scrX*, a gene coding for a protein of unknown function (M_r, 40,000). It is unknown at present whether the product of this gene is a III^{Scr}, perhaps inactive in cells of *E. coli* such that III^{Glc} has to substitute for its activity, or whether it has a function in glucan-fructan biosynthesis. The location of the promoter-operator region was deduced from fusions of the corresponding DNA fragments to the *lacZ* or *galK* genes and to the thermoinducible p_L promoter of bacteriophage λ. Finally, a gene *scrR* was found to code for a repressor (M_r, 37,000). Sucrose and fructose acted as inducers.

In the cariogenic streptococci, for which genetic methods still have to be developed, uptake and metabolism of sucrose through a sucrose-PTS has also been observed. The genes are localized either on the chromosome or on plasmids (355, 361, 369).

(iii) Other enzymes II are found in some but not all strains of the enteric bacteria and in gram-positive bacteria, including those for trehalose (D-glucopyranosyl-α-1-glucopyranoside) (28, 179,221), the ketose L-sorbose (163, 360, 398), and dihydroxyacetone (152). The genes coding for those enzymes II are also located either on the chromosome (Fig. 2) or on metabolic plasmids.

(iv) In the enteric bacteria, lactose, galactose, gluconate, and the pentitols are normally transported in an unphosphorylated form by active transport systems. In most gram-positive bacteria, however, and in a few plasmid-containing strains of the family *Enterobacteriaceae*, these

carbohydrates are taken up and phosphorylated by specific enzymes II. PTS-dependent metabolism of lactose and galactose invariably yields galactose 6-phosphate as an intermediate which, after its isomerization to tagatose 6-phosphate, is metabolized further via the tagatose 6-phosphate pathway (29, 30, 49, 342). Part of this pathway is also involved in the degradation of galactitol and is present in all strains of the enteric bacteria tested thus far (193). Although galactose can be taken up and phosphorylated via II^{Lac}, in many cases a second II^{Gal} is present for this aldose. Both pathways seem to require specific enzymes III, and independent mutations can be isolated for both (52, 53). Only a few genetic studies have been published. These are restricted to the isolation and characterization of mutants. Such mutants either are defective for all PTS carbohydrates and resemble the *pts* mutants from enteric bacteria (89, 112, 253) or are defective in a single PTS activity, owing to inactivation of an enzyme II or an enzyme III activity (206, 207, 242, 243, 245, 353). An exception is the hexitol-specific PTS of *B. subtilis*, which has been analyzed thoroughly (51, 205).

EVOLUTION OF ENZYMES II AND PTS-DEPENDENT METABOLIC PATHWAYS

According to present models of the evolution of genes and polypeptides, new enzymes II and catabolic pathways for PTS carbohydrates might have evolved by gene or operon duplication, followed by mutational diversification of the copies (202, 216, 294). DNA duplication may occur during normal replication or by a different process, characteristic of transposable elements. The latter process involves replication of the transposable element and concomitant translocation of a copy to a new place on the DNA. Such events are frequent within the "collective chromosome" (294, 399) of the procaryotes and are responsible for the exchange of chromosomal and plasmid-encoded genes. The genes for central and vital metabolic functions are located primarily in stable areas of the chromosome. Genes, however, which are responsible for peripheral catabolic pathways or facultative phenotypes are located on plasmids or in regions of the chromosome which exhibit an increased exchange rate of genes with plasmids (6, 356, 360, 399). Typical examples for such facultative phenotypes are the pathways for PTS carbohydrates whose structural genes are located on metabolic plasmids, e.g., enzyme II-dependent pathways for sucrose, sorbose, or lactose in the enteric bacteria and a rapidly increasing number of genes coding for such pathways in the gram-positive bacteria (6, 14, 127, 185, 343, 398). If the genes coding for these pathways are transferred into strains lacking them, they integrate into the chromosome either by recombination between homologous neighboring sequences or by illegitimate recombination. Interestingly, all the genes for PTS carbohydrate metabolism are found within areas of the genome in which large deviations in the order of the genes of *E. coli* K-12, *Salmonella typhimurium*, and *K. pneumoniae* are observed (216, 294). This location in or close to chromosomal rearrangement sites seems to argue for a step-by-step acquisition of new pathways. In certain strains of *E. coli* and *K. pneumoniae*, the *gat* genes for galactitol are replaced in the chromosome by the *atl* and *rtl* genes, responsible for the degradation of D-arabinitol and ribitol, respectively. Both groups of genes exclude each other when in *cis*, although they can coexist in *trans* (210, 399). Sequencing of the corresponding DNA fragments revealed that the *atl-rtl* genes are flanked by 1.4-kilobase inverted repeats of imperfect homology (211). This imperfect homology suggests that the repeats can no longer function in transposition. The *atl-rtl* region can be viewed as the structural remnants of a previously transposable element. After insertion by means of homologous flanking DNA sequence into the *gat* region, the latter must have been deleted. If, in the present state of the genome, the *atl-rtl* or the *gat* genes are transduced, each must necessarily displace the other.

A close relationship at the molecular level is characteristic of genes and metabolic pathways that have evolved by duplication from a common ancestral gene (294). For the 13 enzymes II and related metabolic pathways for PTS carbohydrates described thus far in the family *Enterobacteriaceae*, the following points have been observed.

(i) A similarity at the biochemical level is indicated by the overlapping substrate specificity of many enzymes II (Table 2) and catabolic enzymes. Some enzymes II still share common subunits (e.g., II^{Glc} and II^{Scr} both use III^{Glc}) or HPr. It has even been suggested that II^{Mtl} (M_r, 60,000) originated by a fusion of a smaller enzyme II (M_r, 40,000) with an enzyme III (M_r, 20,000) or that the latter two resulted from the splitting of a large enzyme II. If such an event happened, it cannot have involved II^{Mtl} and III^{Glc}, since these have no immunological resemblance nor any similarity at the DNA level (187, 249).

(ii) The genes coding for enzymes II and the corresponding pathways map at different positions on the bacterial chromosome. Their arrangements within the operons and on the chromosome are highly characteristic (Fig. 2). First, there is a similarity between the hexitol operons, which was mentioned above. A similar arrangement (promoter-proximal gene for the enzyme II, other catabolic enzymes in a distal location) is also found in the *bgl*, *scr*, *sor*, and probably the *nag* operons. Second, the hexitol-specific operons are located between 45 and 81 min, together with the *ptsH,I* and *crr* genes, whereas the genes specific for hexose and hexosamine-PTS are located between 81 and 45 min. There are indications that the present location of *gat* and *fru* might be the consequence of a recent inversion of the part of the chromosome that is located between *gyrA* and *gnd* (18). The uneven distribution of the PTS operons on the genome could be interpreted as relics of an ancient genome duplication (294).

Speculations have been published on how a primitive enzyme II might have evolved. Possibly, a primitive and soluble phosphotransferase molecule or one of the many PEP-accepting molecules underwent fusion at the DNA level to the hydrophobic domain of, e.g., a porin-like protein. The fused protein could have integrated in an asymmetric way in the membrane such that the porin domain faced outward into the medium while the phosphate-accepting domain remained at the cytoplasmic face in a topology similar to that of II^{Mtl} (145, 187). Duplications followed by mutational diversification and step-by-step fusion to other metabolic enzymes and finally by coupling to substrate-specific repressor genes would then have led to the present gene arrangements (191, 202, 360).

REGULATION OF THE PTS

Apart from being an important transport system for many carbohydrates, the PTS is of great importance in the regulation of the peripheral catabolic pathways. It is regulated itself in a highly sophisticated way both at the levels of enzyme synthesis and enzyme activity.

Regulation at the level of enzyme synthesis

In general, enzyme I and HPr are synthesized constitutively, whereas most enzymes II and enzymes III are induc-

ible. If the *ptsHp* promoter is fused to the *lacZ* structural gene, expression of β-galactosidase during growth on PTS and non-PTS carbohydrates is constitutive but at a low level (ca. 1% of the fully induced *lac* operon) (31). With the use of quantitative in vivo transport and in vitro phosphorylation tests or immunochemical methods, however, a two- to fivefold increase in the expression of enzyme I and HPr activity was observed with growth on carbohydrates, with PTS carbohydrates giving the highest levels (226, 292, 363, 387). It is unlikely that these effects correspond to a classical induction, since any PTS carbohydrate increases the activity, whereas repressors are in general very substrate specific. Anaerobic growth conditions seem to favor a high expression (202), in agreement with a hypothesis postulating an important role of the PTS as the major carbohydrate transport system under anaerobic conditions (307). Finally, full expression of the *pts* operon seems to depend on cAMP. *cya* mutants have lower levels of enzyme I and HPr than do wild-type strains (202, 292). A CAP binding site in the *ptsHp* promoter sequence has been found (75a).

The number of enzyme I and HPr molecules per cell has been estimated on the basis of data from rocket immunoelectrophoresis, at 1×10^4 to 3×10^4 molecules of enzyme I and 1×10^5 to 3×10^5 molecules of HPr, respectively (226, 387). The number of HPr molecules seems to be 5 to 10 times higher than the number of enzyme I molecules. It is unclear whether this difference is due to polarity effects inherent in the transcription of the *pts* operon, whether it indicates that in vivo *ptsH* and *ptsI* are transcribed from different promoters (45, 75), or whether it reflects posttranscriptional regulatory mechanisms. Even the lower of the two estimates (enzyme I) is much higher than the number obtained with a *ptsHp-lacZ* fusion strain (31). This discrepancy remains to be explained.

In the few cases analyzed thus far, the inducible enzymes II seem to be controlled in a negative way be highly specific repressor-operator genes and molecules. Thus deletions, Tn*10* insertions, or mutations (including temperature-sensitive ones) of the hexitol-specific regulatory genes result in a constitutive phenotype, and the mutations are *trans*recessive to the corresponding wild-type allele (64, 72, 155, 201). Complementation data of McEntee (227), suggest the existence of a positive regulatory element *srlC* in the *gut* (*srl*) operon. Mutations in the gene *srlC* are pleiotropically negative in the expression of the genes *gutA* and *gutD*, map in or close to the gene locus *gutAp*, and are complemented by λgut$^+$ phages to a normal, inducible phenotype as if they were mutated in an activator gene and under positive control. The *srlC* mutants, however, although negative when induced from outside, become positive when induced from inside (e.g., in the form of 4-*O*-β-D-galactopyranosyl-D-glucitol and uptake through the lactose permease [201]). A similar class of mutants, called *bglS* (283), has been described for the *bgl* operon. This type of suppression argues for a defect in inducer uptake. Such a defect easily simulates the presence of a positive regulatory element in complementation tests, since restoration of transport complements the pleiotropic effect.

Induction of enzymes II can be followed by measuring changes in transport activities or membrane-bound enzyme II activities. Accurate tests require immunochemical methods. When the structural gene for an enzyme II is localized in an operon together with the genes for soluble, catabolic enzymes, the latter genes may be used as indicators, provided no processing of the enzyme II and no drastic polarity effects within the operon exist. With such methods, unexpected differences in the induction patterns of enzymes II have been observed. This is best seen for the two stereoisomers mannitol and glucitol, which, after uptake via the PTS, are converted in one additional metabolic step (catalyzed by the two specific oxidized nicotinamide adenine dinucleotide-dependent dehydrogenases) to the common end product, fructose 6-phosphate. Although II^{Mtl} is induced readily, II^{Gut} is only induced after a lag of 45 to 90 min. This lag seems to be related to poor uptake of the first inducing molecules.

Depending on the growth conditions used, the induced basal level of II^{Mtl} is 5 to 20%, whereas that for II^{Gut} is 0.1 to 2% of the fully induced level. In tight enzyme II-negative mutants and in certain pleiotropic *gut* mutants (*srlC* [227]), the uptake of inducer is so slow that induction from the outside is no longer possible (201). Since such mutants still can be induced if the inducer is brought into the cells by other transport systems, e.g., in the form of disaccharides or trisaccharides through active transport systems followed by hydrolysis via internal hydrolases, we must assume that free intracellular PTS carbohydrates are necessary for induction (162, 201, 343). This result is surprising, since one would expect PTS carbohydrate pathways to be induced either by free carbohydrate in the medium or by the intracellular carbohydrate phosphate.

It has been claimed that the *fru* operon is expressed in a constitutive way in *fruB* mutants that lack fructose 1-phosphate kinase and accumulate fructose 1-phosphate (100, 105). Similarly, the *nag* operon is expressed constitutively in mutants that accumulate *N*-acetylglucosamine 6-phosphate (156). In *S. aureus*, galactose 6-phosphate is thought to be the inducer of II^{Lac} (242). This seems to argue for an induction from within by carbohydrate phosphates, analogous to the well-known endogenous induction of the *gal* operon by intracellular galactose (309). It is, however, not easy to envisage how the cells prevent a permanent endogenous induction, since the carbohydrate phosphates are always present in growing cells (169, 308, 358). For the *uhp* operon, induction from the outside now seems well established (80, 395). A model has been proposed according to which the inducer glucose 6-phosphate binds to the external domain of a transmembrane regulatory protein which forms on the inside of the membrane a complex with an activator protein. Binding of the inducer at the outside is postulated to release or activate the activator at the inside (348). It is tempting to speculate that the enzyme II may itself act in a way analogous to the transmembrane regulatory protein of the *uhp* operon and that binding, translocation, or phosphorylation, or more than one of the above, of a PTS substrate activates transcription. The existence of mutants which can be induced from the inside but not from the outside and the observation that certain enzyme II substrate analogs are not necessarily inducers (191, 201, 363) seem to argue against such a simple model. Obviously, the problem of how and in which compartment induction of PTS-dependent pathways does occur is still unsolved.

Except for the hexitol-specific operons, little is known about how the synthesis of PTS carbohydrate pathways is controlled in mutants that have only one enzyme II for a certain substrate. The general conclusion seems to be that II^{Man} is constitutive in enteric bacteria, that the levels of II^{Glc} vary at most 20-fold in *Salmonella* spp. and *E. coli*, whereas II^{Glc} is constitutive in a few mutant strains (*umgC*) of *E. coli* K-12, and that II^{Fru} is always inducible, as are the other enzymes II. The two- to threefold variations in II^{Man} activity are most probably due not to specific induction but to the

general phenomena of catabolite repression and inhibition. From mutant *E. coli* strains, unable to grow under anaerobic conditions on substrates of II^{Man}, derivatives (*dgsA*) have been isolated with a 10-fold-increased activity of II^{Man}. Since many of these mutants are pleiotropically affected in growth on other carbohydrates too, an unknown type of regulation seems to be involved (301).

The synthesis of the enzymes II is not only controlled by induction but also via catabolite repression. Catabolite repression is known to control the transcription of most catabolic operons in a cAMP-CAP-dependent process (for a review, see reference 375). Usually it is measured in constitutive strains, thus excluding interference by inducer exclusion or expulsion. The enzyme levels in such mutants, pregrown on glycerol or other nonrepressing substrates, are compared with the levels in cells pregrown on repressing substrates. Except for the enzymes II coded for by *ptsG*, *ptsM*, *mtlA*, and *scrA*, all other enzymes II in the enteric bacteria are sensitive to catabolite repression. Even the former enzymes II are not completely insensitive, since expression is lowered in *cya* or *crp* mutants (196, 201, 292). No molecular data are available on this regulation, except for the *mtlA* gene, which has been sequenced. No consensus sequence for a cAMP-CAP binding site was found in the immediate vicinity of the alleged *mtlA* gene locus (187).

A third mechanism, known to control the transcription of most catabolic operons, has been called transient repression. This strong repression is characteristic of cells undergoing a change in the growth rate, such as seen during shift-up experiments or diauxic growth (for a review, see references 258 and 375). In contrast to catabolite (permanent) repression, which lasts for as long as the repressing carbohydrate is metabolized and rarely reaches 80% repression, transient repression, as the name indicates, is relieved after a while, whenever the cells have adapted their metabolism to the new growth conditions. The molecular mechanisms involved are complex and involve a series of processes which differ among various catabolic pathways.

Regulation at the level of enzyme activity

Enzyme II and the general PTS proteins enzyme I and HPr can be considered the pacemaker enzymes in the catabolism of PTS carbohydrates. Similarly to other pacemaker enzymes, their activity is controlled in a complex way.

Enzyme I and HPr. Possible regulation of enzyme I or HPr activity by dimerization has been described previously (180, 235). A different regulation that involves a protein kinase and a protein phosphatase has been described recently in the streptococci (76–79, 285, 286, 288, 289). In these organisms, HPr can be phosphorylated, not only in an enzyme I-dependent reaction at the His-15 residue, but also in an ATP-dependent reaction, involving a protein kinase, at a single seryl residue to form phosphoseryl-HPr. The latter form of HPr is unable to transfer its phosphoryl group via enzymes III and enzymes II to a carbohydrate and is phosphorylated 5,000 times more slowly than is free HPr by phospho-enzyme I. The phosphohistidyl-HPr, in contrast, is a poor substrate for the protein kinase. The kinase does not phosphorylate HPr from enteric bacteria. Initially, a regulation of the protein kinase activity by glycolytic intermediates was described in the streptococci (79). The purified kinase, however, has lost the activation by glycolytic intermediates (77). It has been shown that the very slow phosphorylation of phosphoseryl-HPr by phospho-enzyme I can be stimulated to a different extent by the various enzymes III (78). Addition of III^{Gnd} from *Streptococcus faecalis* restores the rate of phosphorylation of phosphoseryl-HPr to that of free HPr (a 1,000-fold increase). Addition of another enzyme III, III^{Lac} from *S. aureus*, enhances the phosphorylation rate 50-fold compared with that in the absence of enzyme III. These results suggest that the different enzymes III, by complexing with phosphoseryl-HPr, can be phosphorylated with different velocities by phospho-enzyme I. This could result in the preferential uptake of one PTS carbohydrate compared with another and has been observed in *Streptococcus lactis* for 2-deoxyglucose and sucrose (370). A possible role of this modified HPr and a phosphohydrolase in the phenomenon of inducer expulsion was discussed above.

Enzymes II and enzymes III. To distinguish regulatory mechanisms affecting the activity of bacterial transport systems rather than their synthesis, the term catabolite inhibition was introduced (259). Although this term was originally meant to describe an inhibition caused by glucose, it is clear by now that other carbohydrates can cause similar inhibitions and that these inhibitions are due to different control mechanisms.

(i) Substrate competition for a common binding site. Most enzymes II have a broad substrate specificity, binding various substrates with different affinities (Table 2). If two substrates of the same enzyme II are present simultaneously in the medium, they will compete. Depending on the relative affinity constants and the substrate concentrations in the medium, one will eventually exclude the other. This competition is best seen in mutants having only a single enzyme II for both carbohydrates. Thus, in wild-type strains of *E. coli* K-12, mannitol is used preferentially to glucitol, and glucose is used preferentially to mannose or fructose (240) in diauxie experiments (see below). In *mtlA* mutants lacking II^{Mtl}, however, this diauxie is reversed, since mannitol, a low-affinity substrate for II^{Gut} (K_m, approximately 30 mM), is unable to compete with the high-affinity substrate glucitol (K_m, 12 μM) when they are present in equal amounts (199, 202). For similar reasons, mannose or fructose is used preferentially to glucose in *ptsG* mutants of *E. coli*, which lack the high-affinity II^{Glc} system, since their affinities for II^{Man} or II^{Fru} are much higher than the affinity of glucose for these enzymes II (Table 2) (11, 54, 156, 196).

In uptake tests, competitive inhibition of enzymes II starts immediately after the addition of the competing substrate and is maximal from the beginning. It does not require the presence of a second transport system for the competing carbohydrate.

(ii) Inhibition of enzymes II by carbohydrate phosphates. Numerous examples for the regulation of enzyme II activity by carbohydrate phosphates have been given for intact cells, crude cell extracts, and purified membranes or membrane vesicles. In intact cells, this type of inhibition is caused by PTS and even non-PTS carbohydrates, provided they are taken up and metabolized efficiently via a fully induced or constitutive transport system and the corresponding metabolic enzymes. In contrast to competitive inhibition, the inhibiting carbohydrate need not have any affinity for the enzyme II to be inhibited. Inhibition through carbohydrate phosphates generated inside is not maximal from the moment of addition of the inhibiting substrate. Rather, it increases with increasing intracellular carbohydrate phosphate concentrations. The most potent of these inhibitors is glucose 6-phosphate when taken up efficiently via the *uhp*-coded system or when accumulated in mutants with defects in the glycolytic pathway, whereas in in vitro tests, fructose 6-phosphate inhibits most strongly. Nonmetabolizable ana-

logs of PTS carbohydrates which are accumulated in the phosphorylated form in membrane vesicles and cells are strong inhibitors (54, 104, 159, 169, 202, 263, 331).

Using membrane vesicles of *E. coli*, Kaback (159) showed that such a noncompetitive inhibition by hexose phosphates occurs at concentrations of the inhibitor easily reached within cells during shift-up-shift-down conditions, in mutants with a block in metabolism, or after the uptake of nonmetabolizable analogs. Recent results with vesicles of *Salmonella typhimurium* (213) confirmed the finding that methyl α-glucoside phosphate is a feedback inhibitor for II^{Glc} at concentrations above 0.2 mM in the cells. Treatment of the vesicles with toluene relieves this inhibition. This is reminiscent of earlier experiments with mutants of *E. coli* K-12 able to accumulate high intracellular concentrations of glucose 6-phosphate, fructose 6-phosphate, or mannose 6-phosphate. In these strains, several enzymes II were inhibited by the accumulated phosphate esters and the inhibition could be relieved either by inducing the *uhp* transport system or by treating the cells with toluene (80, 104, 169).

The mechanism by which intracellular carbohydrate phosphates inhibit enzyme II activity is unknown. If structurally related to natural substrates of the enzyme II, the inhibitor might interact with the substrate phosphate binding site (303, 323). If it is not structurally related to the product of the enzyme II, an allosteric binding site might be involved.

(iii) Competition between enzymes II for phospho-HPr. Since all PTS carbohydrates use enzyme I and HPr during enzyme II-mediated uptake, they must compete for the general PTS proteins. One can ask whether a cell can catalyze uptake via several enzymes II at the same time and with the same velocity, compared with cells taking up only one carbohydrate, or whether the carbohydrates inhibit each other. Such an inhibition has been observed repeatedly, e.g., between methyl α-glucoside and the hexitols (202), as well as glucose and fructose or *N*-acetylglucosamine (11, 169, 197). A requirement for this type of inhibition is that two different enzymes II must be fully active at the same time.

For nonmetabolizable analogs of PTS carbohydrates, a drastic drain of phospho-HPr, phospho-enzyme I, and PEP (due to continuous dephosphorylation of the carbohydrate phosphate; see above) does occur such that the flow of phosphoryl groups through the PTS becomes the rate-limiting step. If some enzymes II have a higher affinity for phospho-HPr than do others (e.g., II^{Glc}), a decrease in the activity can be predicted and has been measured. From these experiments it has been concluded that enzyme I or HPr is the rate-limiting step in uptake via the PTS (83, 345).

Mutants have been isolated in which glucose no longer excludes fructose or the β-glucosides (11, 93). These mutations map close to the structural gene *fruA* and *bglC*, coding for II^{Fru} and II^{Bgl}, respectively. Although mutations leading to an increased affinity of an enzyme for its substrates are exceedingly rare, the above mutations could have resulted in such an increased affinity of the two enzymes II for phospho-HPr. Alternatively, the enzymes II might have a decreased affinity for the sugar phosphates.

(iv) Regulation of enzyme II by the membrane potential. Early on, it was observed that the addition of an energy source to *E. coli*, which had accumulated methyl α-glucoside to a steady-state level, caused a rapid decrease of the internal glucoside concentration (94, 123, 138, 139, 397). The analog was recovered in the medium in the nonphosphorylated form. The data suggested that the decrease in internal methyl α-glucoside concentration was not due to an inhibition of the influx. Accordingly, an energy-dependent efflux mechanism was postulated. More recent data with inhibitors of respiration or phosphorylation, with intact cells and membrane vesicles, and with mutants affected in respiration or oxidative phosphorylation (109, 124, 125, 137, 165, 363) showed that imposition of a proton gradient across the membrane inhibited methyl α-glucoside accumulation and that an intact respiratory chain, but not ATP, was necessary for this control. Unfortunately, the results obtained by the different groups cannot easily be compared, since some measured predominantly the influx (165, 363), others measured the efflux of the analog (137, 397), and still others measured accumulation of methyl α-glucoside *plus* methyl α-glucoside phosphate (109, 125).

The question has been reexamined recently, by using inverted vesicles capable of maintaining a $\Delta\tilde{\mu}_H+$ (297, 298). It is claimed that in this system, $\Delta\tilde{\mu}_H+$ inhibits II^{Glc} by raising its K_m for methyl α-glucoside by a factor 200 to 1,000. On the basis of these data, a model has been proposed which postulates that II^{Glc} has two substrate-binding sites, one on the outside of the membrane and one on the inside. Furthermore, it postulates that these binding sites have a high affinity for their substrates when in the reduced state, whereas their affinity in the oxidized state is low. Normally, the outside binding site is reduced and binds a substrate. Phosphorylation of II^{Glc} would introduce a negative charge close to the internal binding site, triggering its reduction. As a consequence, the substrate would be drawn to the inside. Transfer of the phosphoryl group from phospho-II^{Glc} to the substrate releases the product into the cell and shifts the equilibrium back to the original state, thus completing the cycle. The model has been applied to a large number of non-PTS transport systems and energy-linked reactions (167). Unfortunately, it is far from having been proved. First of all, it does not explain why in intact cells efflux rather than influx is affected. If, as discussed before, II^{Glc} is located in a strictly asymmetric way in the membrane and if dephosphorylation and efflux are important parameters in the process of methyl α-glucoside accumulation, inverted vesicles might not be the appropriate test system for analyzing the overall reactions. Certainly, data obtained with vesicles, intact cells, or spheroplasts should not simply be compared. Thus, in inverted vesicles the diffusion of methyl α-glucoside into tightly sealed vesicles can be expected to be the rate-limiting step, whereas this step is not necessary in intact cells. A further assumption is that the different inhibitors, e.g., sulfhydryl reagents or uncouplers, interfere specifically and directly with II^{Glc}. This model has been applied to, among others, the lactose carrier (167). Similar experiments were repeated with the lactose permease by using a more sensitive test than was used previously, and it was observed that the loss of transport activity paralleled the loss of binding sites and thus was due primarily to changes in V_{max} rather than changes in the binding constant (251). Recently, this type of experiment has been repeated by Grenier et al. (118) with Enzyme II^{Mtl}. In contrast to the proposal by Robillard and Konings (167, 298), it was concluded that oxidation of II^{Mtl} (and also II^{Glc}) inactivated the enzyme. No change in the affinity was observed.

In conclusion, it appears that control of enzyme II activity is complex and involves a series of different regulatory mechanisms. No molecular details are known for any of these.

REGULATION BY THE PTS

As predicted from the biochemical analysis, mutants defective in an enzyme II or III fail to grow on substrates for

TABLE 3. Phenotype of *ptsH*, *I* and *crr* mutants

Relevant genotype	Growth on[a]:			
	PTS sugars[b]	Class I compounds[c]	Class II compounds[d]	Galactose
Wild type	+	+	+	+
ptsI (leaky)	−	+	+	+
ptsI (leaky) + PT sugar	−	−	−	+
pts(HI)	−	−	−	+
pts(HI) crr	−	+	−	+
*pts(HI) crp**	−	+	+	+
pts(HI) + cAMP	−	+	+	+
crr	+	+	−	+
*crr crp**	+	+	+	+
crr + cAMP	+	+	+	+

[a] +, Growth after 48 at 37°C; −, no growth.
[b] PTS sugars include glucose, mannose, fructose, and hexitols (Table 2).
[c] Class I compounds include maltose, melibose, glycerol, and lactose.
[d] Class II compounds include xylose, rhamnose, and Krebs cycle intermediates, and galactose (via the methyl β-galactoside permease [280]).

this enzyme II unless another transport system and metabolic pathway is available. PTS-negative mutants, defective in enzyme I or HPr or both, in contrast, should be unable to grow on any PTS carbohydrate, i.e., should be pleiotropically negative in their uptake and phosphorylation but should grow normally on non-PTS carbohydrates. This, however, is not the case. Instead, they are unable to grow on a large number of non-PTS carbohydrates, and many have a variable phenotype. We will discuss two major reasons for this diversity.

(i) Existence of suppressor mutations for the PTS functions. Strains of enteric bacteria carrying tight *ptsI* mutations, e.g., Tn insertions or deletions, do not grow on any PTS carbohydrate or on a number of non-PTS carbohydrates (Table 3), whereas tight *ptsH* mutants still grow on the PTS carbohydrate fructose. After pregrowth on this ketose and to a lesser extent on glucose or mannose, *ptsH* mutants grow for a limited time on other PTS carbohydrates (332), apparently because an HPr-like activity is activated (induced?) under these conditions. As discussed before, the proteins involved could be FPr or other proteins inducible (activated) by fructose (128, 379, 386). After removal of the inducer from the medium, the cells grow at the expense of this HPr-like activity until it is diluted out or degraded. All *ptsH* mutants described thus far can mutate at a very high rate to a Pts^+ phenotype without regaining HPr. These suppressor mutations map outside the *pts* region and cause the constitutive expression of a pseudo-HPr activity, most probably the HPr-like or FPr activity mentioned before (62, 332, 333, 386). Although the precise relationship between the different proteins inducible by fructose, the pseudo-HPr, and the HPr-like activities remains to be established, the unusually high mutation rate of *ptsH* mutants to the Pts^+ phenotype suggests that these mutations cause the constitutive expression of a protein(s) inducible or activated by pregrowth on fructose (and other hexoses) in the wild type.

A series of other suppressor mutations allow growth of tight *ptsI* mutants on some but not all PTS carbohydrates. In general, they open up a PTS-independent transport system or metabolic pathway or both for one or more PTS carbohydrates. Thus, the enteric bacteria exhibit a Glc^+ phenotype if one of two galactose permeases (coded for by the genes *galP* or *mglA,B,C*, respectively) mutate to constitutive expression (198, 272, 315). Both permeases are inducible by galactose but have a high affinity for glucose and are active transport systems whose energization is independent of the PTS. The suggestion that in *E. coli* K-12 the major glucose transport system is anyway a non-PTS transport system (377) was shown to be incorrect (277). Free internal glucose is phosphorylated subsequently by a soluble, ATP-dependent kinase coded for by the gene *glk* (65). The same mutations also allow growth on fructose and mannose if an ATP-dependent manno(fructo)kinase is expressed at high levels as a result of mutations in an unmapped gene locus *mak* (336). No such suppressor mutations are known for the three hexitols mannitol, glucitol, and galactitol in wild-type strains of *E. coli* K-12 and *Salmonella typhimurium* (191). In *K. pneumoniae*, however, and in enteric bacteria able to grow on D-arabinitol, a constitutive expression of the corresponding active transport system and dehydrogenase suppresses tight *ptsI* or *mtlA* mutants to a Mtl^+ phenotype, since the arabinitol transport system also accepts mannitol and the dehydrogenase converts mannitol to fructose (192, 366).

An unexpected pathway for glucose metabolism in *K. pneumoniae* was described recently (252). It involves a quinoprotein glucose dehydrogenase that uses 2,7,9-tricarboxy-1*H*-pyrrolo-(2,3-f)quinoline-4-5-dione (PQQ) as its prosthetic group. This dehydrogenase converts glucose to gluconate. The presence of this efficient pathway may explain the early observation (367) that *ptsI* mutants of *K. pneumoniae* still grow on glucose (at one-third the maximal rate), whereas *ptsI* strains of *E. coli* or *Salmonella typhimurium* do not. The glucose dehydrogenase apoenzyme is also synthesized in *E. coli* and *Salmonella typhimurium*, but these organisms are unable to synthesize PQQ. *pts* mutants of *E. coli* and *Salmonella typhimurium* can grow on glucose if PQQ is supplied (140).

There are thus several ways in which *pts* mutants can grow on PTS carbohydrates, provided a non-PTS pathway can be created. Although this complicates the study of such mutants and their derivatives, new pathways can be discovered in this way.

(ii) Suppressor mutations allowing the expression of non-PTS carbohydrate metabolic pathways in *ptsH,I* mutants. The phenotype of *pts* mutants is more comprehensive than we have discussed till now. The earliest *ptsH* and *ptsI* mutants isolated in enteric bacteria or in *S. aureus* were described as pleiotropically negative in fermentation of most carbohydrates. One of the puzzling features of such mutants was the extent of the pleiotrophy, which included carbohydrates, like galactose, maltose, lactose, melibose, glycerol, xylose, ribose (all known to be non-PTS carbohydrates), the tricarboxylic acid cycle intermediates, and the amino acids L-tryptophan and D-serine. Very early, it became clear from the genetic data that the failure to grow on non-PTS carbohydrates was not directly related to the lack of some PTS functions, since suppressor mutations, restoring growth on non-PTS carbohydrates, did not restore the PTS activities. Rather, the inability of *ptsH,I* mutants to synthesize inducible catabolic transport systems and metabolic pathways was responsible for this phenotype.

Catabolite repression and inducer exclusion

To understand at the molecular level why *ptsH,I* mutants are unable to induce a series of non-PTS catabolic pathways, a description must be given of several regulatory processes, discovered by Epps and Gale (95) and Monod (240).

When bacteria are exposed to two different carbon sources, one is frequently used preferentially and growth occurs in two phases (diauxie). As suggested originally by Monod (240), most substrates, which he called class B carbon sources (e.g., glucitol, fructose, galactose, maltose, lactose, or glycerol) are excluded from cells during the first growth phase of such a diauxic growth by class A carbon sources. For the enteric bacteria, the best known class A substrate is glucose; hence the name glucose effect to describe this exclusion phenomenon. Other class A substrates are mannitol, *N*-acetylglucosamine, gluconate, or glucose 6-phosphate. Subsequent work by Cohn and Horibata (56–58) and other groups showed that glucose and the other class A substrates prevent the uptake of the first inducing molecules of any class B substrate by decreasing the activity of the corresponding transport systems or of the first metabolic enzyme (inducer exclusion).

Neidhardt and Magasanik (217) showed subsequently that the glucose effect was not restricted to glucose and other class A substrates, but could even be caused by any class B substrate, provided that the rate of its catabolism surpassed the rate of anabolism, e.g., under the conditions of phosphate or nitrogen limitation. In mutants with completely derepressed metabolic enzymes for a class B substrate such as glycerol (23, 209) the latter also caused a strong glucose effect. Finally, when the class A substrates glucose or mannitol were forced to enter the cells through the transport systems for the class B substrates fructose or glucitol, respectively, they behaved like class B substrates (11, 169, 199). According to the original hypothesis, any growth conditions which causes an excess of catabolism over anabolism will cause the intracellular accumulation of a catabolite repressor. This general repressor was postulated to be responsible for the inhibition of transcription of catabolic operons. Catabolite repression requires either the rapid uptake and continuous metabolism of a carbon source or a normal metabolism coupled to a reduced anabolism. It rarely exceeds 50 to 80% repression (217, 218, 259). Under the conditions of a shift up or during diauxic growth, another, more drastic (80 to 100%) repression can be observed (258, 259). By contrast to the permanent or catabolite repression, which lasts for as long as the repressing compound is present, this transient repression lasts only from 0.1 to 1.0 doubling times. It may also be generated by substrate analogs which cannot be metabolized beyond the first phosphorylation steps (218, 258, 259, 374).

In general, operons coding for peripheral catabolic pathways are regulated at the level of transcription by an operator and a repressor or an activator. Inactivation of the repressor (negative control) or activation of the activator (positive control) by an inducer triggers induction, a process specific for a single operon or a regulon. Induction alone, however, is not sufficient to allow the full expression of catabolic operons. Their promoters often require the presence of a complex between a protein, called CAP (43) or CRP (product of the gene *crp* [261]) and cAMP. Different promoters require different amounts of the cAMP-CAP complex (9, 166, 212). Mutants lacking CAP or cAMP, owing to mutations in the gene *crp* or *cya* (the structural gene for adenylate cyclase), are pleiotropically negative for all operons requiring cAMP-CAP. The effect of this complex on transcription is not fully understood. The evidence has been reviewed recently (38, 261, 375).

Exogenous cAMP can overcome catabolite repression completely and transient repression partially (259, 261, 262). This was taken as an indication that in both processes a modulation of the cAMP-CAP activity and eventually of the transcription rate is involved. The hypothesis seems to be supported by numerous measurements of the level of intracellular and extracellular cAMP under conditions of high or low catabolite repression and in mutants containing various alleles of the genes *cya* and *crp* (40, 84, 97, 120, 157, 219, 248, 267). Both types of mutants show a severe and permanent catabolite repression which can be suppressed in *cya* mutants by exogenous cAMP but not in *crp* mutants. The *cya* mutants can also be suppressed by *crp** mutations (337), which alter CAP in such a way that it is active in promoting transcription in the absence of cAMP. Some *crp** alleles allow growth on only a few compounds, whereas others restore growth on most carbon sources. Lis and Schleif (212) and others (9, 166) have shown that some promoters require less activated CRP for a full transcription rate than others. The variable phenotypes of *crp** mutants probably reflect different activity of the altered CAP protein. All *crp** mutations are pleiotropic and affect several operons. One can also isolate suppressor mutations that restore growth of *cya* or *crp* mutants on a single carbon source. They allow a cAMP-CAP-independent transcription of a single operon, e.g., by altering the promoter. Although there is little doubt that cAMP-mediated repression and, to a lesser extent, transient repression affect the transcription of catabolite operons by modulating the intracellular level of the cAMP-CAP complex, any simple model postulating a direct relationship between the level of this complex and the degree of repression clearly is insufficient to explain all the available data (375, 384).

As postulated by Monod (240) inducer exclusion is responsible for the exclusion of a class B substrate from the cell by a class A substrate. Although catabolite and transient repression play an important role in this exclusion process by inhibiting the synthesis of the corresponding transport systems, they are not necessarily the most important control mechanisms. Instead, for many cells and for many catabolic pathways, inducer exclusion is caused primarily by inhibition of the activity of the transport system for the class B substrate by the class A substrate or by one of its metabolic derivatives. Several mechanisms are responsible for this phenomenon of inducer exclusion. (i) The first is competitive inhibition, i.e., direct competition of the two carbon sources for a common transport system. It is most easily observed when two substrates with similar affinities for a particular transport system compete under conditions when no other transport system is available for one or both of these substrates. An example is the competition of glucose and galactose for the galactose permease (1, 190). Competitive inhibition is frequently the major regulatory mechanism in diauxie and reversed diauxie (11, 199). (ii) The second is allosteric interaction by low-MW effector molecules, e.g., carbohydrate phosphates, as discussed above. (iii) The third is competition between enzymes II for a common intermediate, e.g., phospho-HPr (345). (iv) The fourth is inhibition of non-PTS uptake systems by interaction with III^{Glc} of the PTS. A detailed description of this important regulatory mechanism is given below. (v) For most catabolic operons and regulons, the substrate of the first metabolic enzyme is the inducer, and inducer exclusion is caused by repression and inhibition of the corresponding transport system. In a few cases, however, such as the *lac* operon or the *glp* operon, the product of the first enzyme is the inducer. In the first case, the lactose permease is regulated, but in the second example the glycerol facilitator is not affected. Instead, the synthesis of the inducer glycerol 3-phosphate is

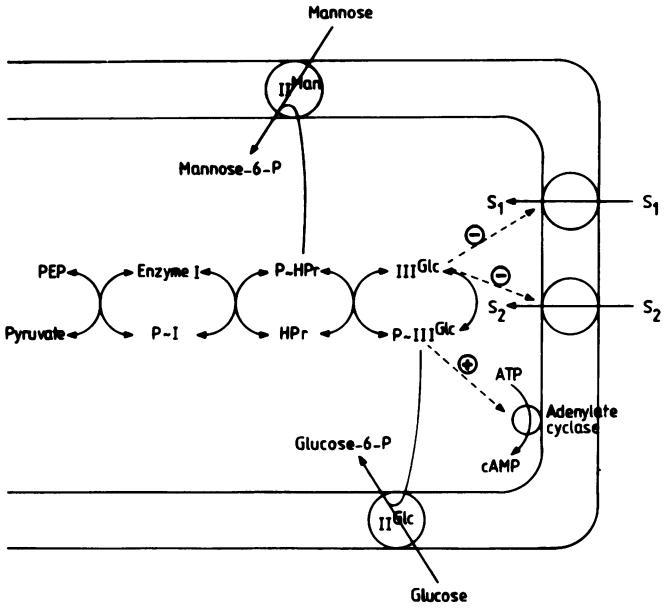

FIG. 3. Model for the regulation by the PTS. In addition to the general proteins of the PTS, two enzymes II are shown, specific for mannose (II^{Man}) and glucose (II^{Glc}). Activation (+) of adenylate cyclase by phosphorylated III^{Glc} ($P\sim III^{Glc}$) and inhibition (−) of two different non-PTS uptake systems by III^{Glc} are indicated. S_1 and S_2 represent lactose, melibose, maltose, or glycerol.

regulated by fructose 1,6-bisphosphate at the level of the first enzyme, glycerol kinase (209). Since in the regulation of glycerol kinase III^{Glc} again plays a central role, it is discussed in more detail below.

Role of the PTS in catabolite repression and inducer exclusion

In members of the family *Enterobacteriaceae* the PTS plays a central role in catabolite and transient repression as well as in inducer exclusion. Four important observations lie at the basis of our present understanding of the molecular mechanisms that are involved in the control exerted by the PTS on other transport systems and on the expression of peripheral catabolic pathways.

(i) A close connection between glucose uptake and the various glucose effects had long been noted. Thus, most of the mutants resistant against the glucose effect were found to be defective in the glucose transport system now known to be II^{Glc} (17, 41, 94, 262, 374, 376). At least in the case of *E. coli* K-12, all mutants showed a reversed diauxie, i.e., glucose is no longer taken up in preference to other carbon sources (for a review see reference 218).

(ii) Tight *ptsH,I* mutants were found to have a similar phenotype as *cya* or *crp* mutants, i.e., they are impaired in the induction of catabolic transport systems and operons. In this respect they resemble wild-type cells, which in the presence of glucose or other class A substrates are also unable to induce catabolic operons for class B substrates. Furthermore, exogenous cAMP was found to overcome the defects of tight *ptsH,I* mutants (262). Finally, the same suppressor mutations which had been found to suppress the defects that result from *cya* or *crp* mutations or both were found to suppress tight *ptsH,I* mutations too. These included the pleiotropic *crp** mutations (8, 279, 344) as well as specific regulatory mutations that allow cAMP-CAP-independent transcription of single catabolic operons (24, 121, 248, 335, 382). Interestingly, these suppressors also included mutations that result in the constitutive expression of an operon (17, 198, 315, 382).

(iii) The decisive clue both to a central role of the PTS in the regulation of transport systems and catabolic pathways and to the molecular mechanisms involved was provided by a new type of suppressor mutation, first isolated in *Salmonella typhimurium* cells (326, 327) and later also in *E. coli* K-12 cells (50, 173, 174). This suppressor allowed growth of tight *pts* mutants on a series of non-PTS carbohydrates such as maltose, melibiose, glycerol, and lactose. The suppressor mutation was called *crr* for carbohydrate repression resistance, an unfortunate name which suggested a possible identity between this PTS-mediated repression and catabolite repression. As discussed before, *crr* has been shown in the meantime to be the structural gene for the soluble III^{Glc}.

(iv) Final evidence was obtained from studies with leaky *ptsI* mutants (<1% residual activity), still able to grow on the non-PTS carbohydrates just mentioned. Addition of any PTS carbohydrate or one of its nonmetabolizable analogs to these leaky *ptsI* mutants inhibits growth on the non-PTS carbohydrates completely (326, 328). This inhibition was shown to be due to a severe transport inhibition, a noncompetitive inhibition seen only when the enzyme II, specific for the inhibitor, was present. Mutants resistant against this inhibition were found to lack III^{Glc}, the mutation mapping in *crr*. The term inducer exclusion was introduced to describe this inhibition of non-PTS transport systems by PTS carbohydrates, although the name originally meant any process interfering with inducer uptake or synthesis or both (218, 259). To differentiate the present process from others and to indicate the central role of III^{Glc}, it will be called III^{Glc}-mediated inducer exclusion.

On the basis of these and other results, a model for regulation was advanced (278, 317, 327, 328), in which III^{Glc} plays a central role which is summarized schematically in Fig. 3. As discussed above, all PTS proteins, including III^{Glc}, can be phosphorylated and dephosphorylated. The phosphorylation level of III^{Glc} depends on the influx of phosphoryl groups through PEP, enzyme I, and HPr and on the efflux via II^{Glc} to the substrate phosphate. Tight *ptsH,I* mutants contain only nonphosphorylated III^{Glc}. In leaky *ptsI* mutants, however, III^{Glc} is phosphorylated as long as no PTS carbohydrate(s) is taken up. Although substrates of II^{Glc} can dephosphorylate III^{Glc} directly, all other PTS carbohydrates can do so indirectly by trapping phospho-HPr and reversing the equilibrium between phospho-III^{Glc} and HPr (reaction 3b). Central to the model are two reactions of III^{Glc}: (i) phospho-III^{Glc} activates adenylate cyclase and thus regulates the transcription of catabolic operons and the genes coding for transport systems by modulating the level of intracellular cAMP and activated CAP; (ii) nonphosphorylated III^{Glc} is an inhibitor of a number of non-PTS uptake systems, permanently inactive in tight *ptsH,I* mutants, and (transiently) inactive in leaky *ptsI* mutants as long as PTS carbohydrates are present.

Interaction between adenylate cyclase and III^{Glc}

Although the similarity in the phenotype of tight *ptsH,I* mutants, *cya* and *crp* mutants, and wild-type strains under conditions of catabolite or transient repression suggested a close linkage between their phenotypes and the level of intracellular cAMP-CAP, the molecular basis of this connec-

tion was unclear. An important discovery was made by Peterkofsky and coworkers (131, 266–270), who found that the activity of adenylate cyclase, measured either in intact cells or in cells permeabilized by toluene, was inhibited by the addition of any PTS carbohydrate, provided that the corresponding enzyme II was active (130, 318). At first it was concluded from work with different *pts* mutants of *E. coli* that phospho-enzyme I activates the cyclase, either by phosphorylating the enzyme or by forming an active complex with it. Later studies showed, however, that *ptsH* mutants lacking HPr or *crr* mutants defective in III^{Glc} but with normal levels of enzyme I also have lowered adenylate cyclase activity (102, 248).

The basal level of adenylate cyclase in tight *crr* mutants is sufficient for growth on some non-PTS carbohydrates (class I in Table 3), but not for growth on other compounds (class II in Table 3) (274). Similarly, tight *ptsH,I* mutants that have acquired a *crr* mutation regain the ability to grow on class I but not class II compounds, whereas leaky *ptsI* mutants, containing an additional *crr* mutation, can grow on class I substrates in the presence of PTS carbohydrates but not on class II substrates (333, 344). This difference between class I and class II compounds is most probably due to the presence of low, residual levels of cAMP in *pts crr* double mutants. These low cAMP levels are sufficient to allow the transcription of the promoters of the catabolic operons for class I compounds, but not of the promoter for class II compounds. Growth of *pts* or *crr* strains on class II substrates is restored by exogenous cAMP or the introduction of a *crp** allele in the cells (8, 280, 344). On the basis of these and other data, it has been postulated that phospho-III^{Glc} is an activator of adenylate cyclase.

It has been observed repeatedly that *crp* mutants lacking CAP have increased levels of adenylate cyclase activity and secrete cAMP into the medium (40, 119, 219, 278, 375). Although several lines of evidence seem to indicate that this is due to an increased transcription of the *cya* gene locus with CAP possibly acting as a repressor (40), other data suggest that the activation is primarily at the level of the enzyme molecules (19). The existence of an inhibitor of adenylate cyclase has been suggested from data on the purification of this enzyme (401). Dobrogosz and co-workers have also postulated from mutant studies that CAP might form a complex with adenylate cyclase and inhibit its activity (84, 129). The cell contains a limited number of CAP molecules (122). On the basis of studies on the expression of the *lac* operon in various *pts* mutants, Gershanovitch and coworkers postulated a direct coupling between CAP and the PTS which modulates transcription of catabolic operons without the involvement of cAMP (36, 116). They found that in a *cya ptsI* strain the *lac* operon was repressed even in the presence of exogenous cAMP. The same *pts* mutation had no effect in *crp* mutants or in mutants with an altered *lacZp* promoter, independent of cAMP-CAP for full transcription.

At this moment we are unable to tell whether III^{Glc} activates adenylate cyclase directly (possibly by phosphorylation) or indirectly by eliminating an inhibitor (CAP) (see also references 69 and 376). It should also be noted that catabolite repression is still possible in *ptsI* strains (400). This again points to the fact that not all repression can be due to regulation of adenylate cyclase activity by III^{Glc}, since in these mutants no alteration in the phosphorylation state of III^{Glc} is possible. Proof of any molecular model must await the purification of the enzyme in an active form which can still be regulated.

Adenylate cyclase from *E. coli* K-12 has recently been purified to near homogeneity (M_r, 92,000 to 95,000 [401]). It is catalytically active as the monomer, and only a few (approximately 15) such catalytically active enzyme complexes are found in the cell. Danchin et al. (68) concluded, however, from studies with a fused adenylate cyclase-β-galactosidase hybrid that wild-type *E. coli* cells contain approximately 1,000 adenylate cyclase molecules. Contrary to previous reports (219, 368) suggesting a membrane location, it was found that adenylate cyclase was predominantly a soluble protein, perhaps loosely bound to the membrane (401). Alternatively, the enzyme might be bound in its active, intact form to the membrane and released in soluble and catalytically inactive form by proteolysis during cell fractionation. This possibility is considered unlikely by Yang and Epstein (401). Several observations remain to be explained, however. (i) Adenylate cyclase activity was found either in the pellet or in the supernatant fractions, depending on the methods used for cell disruption and fractionation. This activity was very low compared with the activity in intact cells or in toluene-treated cells (40, 131, 151, 219, 267), and decreased nonlinearly on dilution, perhaps indicating the dissociation of a protein complex (401). (ii) Only intact cells or cells permeabilized by toluene treatment retained the regulation of adenylate cyclase activity, as if a regulatory protein or subunit dissociates irreversibly on dilution (131). (iii) The *cya* region of *E. coli* and *Salmonella typhimurium* has been cloned, and the gene from *E. coli* has been sequenced (5, 68, 168, 189, 380). The corresponding DNA fragments code for two proteins (M_r, 92,000 and 32,000) and possibly for a third (M_r, 37,000), the larger being sufficient to complement *cya* mutants for the production of cAMP, i.e., the catalytic activity of adenylate cyclase. The NH_2-terminal part of the purified enzyme corresponds to the DNA sequence which starts in both cases with UUG instead of the usual AUG triplet (68). Deletion of large parts of the 3′ end (ca. 1.4 kilobases) of the *cya* gene did not affect the catalytic activity of its product, perhaps indicating that this part of the molecule is not involved in catalysis but in the regulation of its activity (189). According to Roy et al. (310), glucose inhibits the intact protein but not a truncated enzyme that has lost its carboxy terminus. This important question is unresolved, since another group reported that such truncated molecules are still regulated normally by glucose as measured by intracellular cAMP levels and *lac* operon expression (168). Whether one or both of the additional proteins, coded for in the *cya* region, are involved in the regulation of adenylate cyclase activity or synthesis or both remains to be shown.

Other factors can also regulate adenylate cyclase activity, although probably in an indirect way. These include the electrochemical proton gradient (266, 270), ppGpp (an alarmone [9] for anabolic pathways), and 2-ketobutyrate, an analog of pyruvate, known to react with phospho-enzyme I. In the presence of functional enzyme I, HPr, III^{Glc}, and CAP molecules, accumulation of 2-ketobutyrate causes a drastic inhibition of adenylate cyclase, most probably by the mechanism discussed before (formation of nonphosphorylated III^{Glc}) (67, 70). Such a feedback between the PTS and the oxidation state of the cell seems reasonable.

Obviously, then, it is not known at present how many regulatory mechanisms exist which control the synthesis and activity of adenylate cyclase and whether III^{Glc} acts directly in the phosphorylated form by activating the enzyme through phosphorylation or whether it interferes with an inhibitor, possibly CAP.

If cAMP-mediated catabolite and transient repression are

related to the intracellular pool of cAMP (and thus activated CAP), any mechanism controlling this pool must be relevant to our understanding of this highly complex phenomenon. The internal pool of cAMP depends not only on its rate of synthesis but also on its rate of breakdown by phosphodiesterase (38, 39) and on efflux from the cells by an energy-dependent process that is stimulated by metabolizable carbon sources (63, 117, 319, 375).

Interaction between non-PTS uptake systems and III^{Glc}

As a possible explanation for the glucose effect and diauxic growth, Epps and Gale (95) and Monod (240) put forward the hypothesis that class A substrates such as glucose inhibit the uptake of non-PTS carbohydrates belonging to class B. Cohn and Horibata (56–58) and later Clark and Marr (55) showed that the degree of this inhibition was dependent on the concentration of both the inhibitor and the class B substrate in the medium as well as on the level of the transport systems for both compounds. They suggested a possible stoichiometric interaction between the two transport systems. In the case of the galactose system, a direct competition between glucose and galactose or the gratuitous inducer D-fucose was shown to lie at the basis of inducer exclusion (1, 190). Winkler and Wilson, studying the effect of methyl α-glucoside on β-galactoside transport via the lactose permease concluded that in cells containing both transport systems the glucoside noncompetitively inhibited the uptake of the nonmetabolizable lactose analog TMG, whereas this galactoside did not inhibit uptake of methyl α-glucoside (397). Similar studies conducted in great detail by Saier and coworkers (50, 83, 326–328, 332, 333) confirmed previous observations that in tight *ptsI* mutants of *Salmonella typhimurium* a series of non-PTS catabolic pathways could not be induced. They observed, however, that leaky *ptsI* mutants could still be induced. The synthesis of the non-PTS pathways was sensitive to repression by low concentrations of any PTS carbohydrate, provided that a functional enzyme II for the latter was present in the cells. This repression was shown to be due, at least in part, to a noncompetitive inhibition of the non-PTS uptake systems. Among the mutants which had lost this repression by PTS carbohydrates were found a new class called *crr* (carbohydrate repression resistant), lacking III^{Glc} (see above). These data led to the present model of PTS-mediated regulation.

On the basis of additional and recent reconstitution experiments with purified III^{Glc}, the purified lactose carrier reconstituted in liposomes, and glycerol kinase, a mechanistic model can now be presented (Fig. 3) which explains at a molecular level the III^{Glc}-mediated inhibition of several non-PTS uptake systems such as those for lactose and glycerol and probably also for maltose and melibiose. The following results were obtained (250): (i) Purified III^{Glc} binds to membranes from strains of *E. coli* that contain the lactose permease in high amounts as a result of the presence of a high-copy-number plasmid into which the *lacY* gene has been cloned. The binding of III^{Glc} requires the presence of a substrate of the lactose permease. This binding was first described by Osumi and Saier (257). The corresponding K_D range from 5 to 15 μM; 1 to 1.5 molecules of III^{Glc} (M_r, 20,000) were bound per molecule of lactose permease (M_r, 45,000).

(ii) Phosphorylation of III^{Glc} prevented its binding to the permease. (iii) In liposomes reconstituted with purified lactose permease, the apparent K_D for *o*-nitrophenyl-α-galactoside decreased in the presence of III^{Glc} from 22 to 7 μM. (iv) III^{Glc} does not bind to a mutant lactose carrier, containing three amino acid changes in the NH_2-terminal part of the molecule. (v) In membrane vesicles and in liposomes reconstituted with the purified lactose carrier, III^{Glc} inhibits the energy-dependent thiodigalactoside countertransport, the lactose-lactose equilibrium exchange, and active transport supported by an artificial ion gradient. No other proteins are required for this inhibition, which in intact cells prevents uptake of β-galactosides and causes inducer exclusion.

These data strongly support the model summarized in Fig. 3, as far as III^{Glc} and the *lacY*-coded lactose permease are concerned. The inhibition, however, is not restricted to this system. Glycerol uptake is also sensitive to III^{Glc}-mediated inhibition (328). This uptake first involves the translocation of glycerol through the membrane by means of a facilitator which seems to act as a pore (209). Glycerol is converted by glycerol kinase to glycerol 3-phosphate, the first metabolic intermediate and at the same time the inducer of the *glp* regulon. It was shown recently that the activity of glycerol kinase is inhibited by III^{Glc}, whereas phospho-III^{Glc} has no effect (276). Since glycerol 3-phosphate is the real inducer, inhibition of its synthesis again leads to III^{Glc}-mediated inducer exclusion.

These reconstitution experiments have given conclusive evidence for the hypothesis that III^{Glc} directly regulates the activity of several uptake systems (or closely related metabolic enzymes) and their induction.

Interestingly, Reizer et al. (287) recently described inhibition of glycerol uptake by methyl α-glucoside in *B. subtilis*. Earlier studies by Gay et al. (112) had shown already that *ptsI* mutants of *B. subtilis* were unable to grow on glycerol. Possibly a molecule analogous to III^{Glc} in enteric bacteria plays a role in regulation of glycerol uptake in *B. subtilis*.

III^{Glc}-mediated regulation in intact cells

III^{Glc} not only interacts with various membrane-bound non-PTS transport systems and with II^{Glc} and II^{Scr} but also with the soluble proteins phospho-HPr, glycerol kinase, and possibly adenylate cyclase. In *pts* mutants, most of the III^{Glc} is present in the nonphosphorylated form. In wild-type cells, however, its phosphorylation becomes the rate-limiting step whenever a PTS carbohydrate is taken up and phosphorylated effectively by an enzyme II. According to the proposed model (Fig. 3), free III^{Glc} interacts with most of the proteins mentioned above, whereas phospho-III^{Glc} interacts with adenylate cyclase and II^{Glc}. Consequently, it should be possible to titrate out the III^{Glc} molecules in intact cells by increasing the amount of one of these proteins or alternatively to saturate them by overproducing III^{Glc}.

The amount of III^{Glc} in *Salmonella typhimurium* cells, as measured by immunological methods, is relatively constant under all growth conditions (346). If, as postulated by the model, a stoichiometric complex is required between III^{Glc} and the target protein, one can predict that a certain percentage of the transport system molecules will escape from inhibition under conditions of full induction if their number exceeds the number of III^{Glc} molecules. In agreement with this, wild-type cells, fully induced for the *lac*, *mal*, or *glp* systems, are resistant to III^{Glc}-mediated inducer exclusion, although the exclusion is still observed in cells partially induced for these systems (239, 247, 256, 322). Mutants with lowered levels of III^{Glc} escape from such an inhibition more easily (248, 274), whereas cells of *Salmonella typhimurium* carrying a multicopy plasmid, containing the *crr* gene, and

overproducing III^{Glc}, can become hypersensitive (247) to repression by glucose and inducer exclusion of sensitive, non-PTS metabolic pathways. Overproduction of III^{Glc} from a multicopy plasmid has been claimed to be harmful in *E. coli* cells and to render such cells unable to utilize a wide variety of carbohydrates (230). This is not observed with a plasmid carrying the *crr* gene from *Salmonella typhimurium* (249). One would expect no effect as long as III^{Glc} can be kept phosphorylated, e.g., in the absence of PTS carbohydrates.

A prediction of the model is that when two target proteins are present at the same time in high concentrations, they might compete for III^{Glc}. This has indeed been observed (247), e.g., when both the maltose and the glycerol systems of *Salmonella typhimurium* were induced. In such cells, the presence of glycerol renders the maltose system less sensitive to inhibition by PTS carbohydrates. Saier et al. reported similar findings in a strain overproducing the lactose permease (325). In these strains, thiodigalactoside released the glycerol and maltose uptake systems from inhibition by methyl α-glucoside and other PTS carbohydrates.

Leaky *ptsI* mutants, normally hypersensitive to inhibition by PTS carbohydrates, can be rendered resistant to this inhibition by introducing a *crr* mutation or a *crp** mutation (279, 344) but also by growing such cells in the presence of external cAMP (279, 322, 344) or high concentrations of an inducer and by mutations allowing the constitutive expression of the sensitive non-PTS transport systems. Although it was suggested that this reflects a different regulatory mechanism (322), in our opinion each of these suppressor conditions tends to increase the number of sensitive transport systems molecules above the number of III^{Glc} molecules (247), and therefore supports the concept of a single regulatory mechanism.

Additional suppressor mutations allowing growth of *pts* mutants on non-PTS compounds

Tight *ptsI* mutants are unable to grow on many non-PTS compounds. For full expression, all sensitive operons require high internal concentrations of the inducer and of cAMP, both of which are regulated by the PTS and III^{Glc}. It was suggested that some catabolic operons (class I in Table 3) require less cAMP than others (class II, [9, 212]). Variable affinities of different promoters for activated CAP were measured recently (166). This requirement is also reflected in their variable response to suppressors. (i) Growth of tight *ptsI* mutants on the non-PTS carbohydrates grouped in class I (Table 3) is restored by a *crr* mutation, whereas it renders *ptsI* mutants resistant to inhibition caused by PTS carbohydrates. (ii) Growth of such *pts* mutants on non-PTS compounds grouped in class II is only restored by exogenous cAMP or introduction of a *crp** mutation (8, 344). Obviously, their need for activated CAP is so high that it cannot be compensated for solely by high internal inducer concentrations. (iii) Among the earliest suppressor mutations isolated were ones that restored growth of *pts* mutants on a single carbon source without restoring the missing PTS functions. These included mutations in the repressor gene for the *lac* operon (17, 382), the *glp* regulon (24), or the *gal* and *mgl* operons (198, 315), causing constitutive expression of these systems, or in promoter-up mutations, with the same effect (25). Interestingly, one of the earliest leaky *pts* *E. coli* mutants isolated (MM6 [17]) and recognized as such (365), also contained a *lacI* mutation and a mutation, originally called *suc-1* (7, 8), that is most probably a *crr* mutation. High concentrations of the nonmetabolizable inducer isopropyl-β-D-thiogalactoside in the case of the *lac* operon (261), and high concentrations of glycerol 3-phosphate in the case of the *glp* regulon (23) can also be used to stimulate induction of these systems.

In addition to the *glpR* and the *glpKp* mutations, a third type of suppressor for the expression of the *glp* regulon was isolated in the structural gene for glycerol kinase, which renders it insensitive to feedback inhibition by fructose 1,6-bisphosphate (23). Berman and Lin (23) suggested that the lowered amount of glycerol kinase in *ptsI* mutants was not sufficient to allow an efficient synthesis of the inducer glycerol 3-phosphate unless any feedback inhibition of the kinase was relieved. Whether the phenomenon is related to the inhibition of the kinase by III^{Glc} remains to established. It is possible that this mutation renders glycerol kinase insensitive to inhibition by III^{Glc} at the same time.

Other specific suppressor mutations have been located close to or in the *malB* region, the *glpK*, the *lac*, and the *mel* operons of *Salmonella typhimurium* and *E. coli* (335) and were found to render a specific transport system resistant to regulation by PTS carbohydrates. The experiments do not show unequivocally, however, whether this resistance is at the level of the synthesis of the transport systems or its activity, or both. If the mutations are in the structural genes, the mutants might synthesize altered transport systems insensitive to III^{Glc} inhibition.

If the regulation of adenylate cyclase by the PTS is also due to an interaction between this enzyme and III^{Glc}, suppressor mutations mapping in the *cya* locus might exist which uncouple adenylate cyclase from the regulation exerted by phospho-III^{Glc}. Such mutations, allowing growth of tight *ptsI* mutants on all class I non-PTS carbohydrates, have been isolated (274) but not yet characterized in molecular detail.

Final and unexpected support for the present model on regulation came from the analysis of a new class of suppressor mutations called *iex*, characterized by Kornberg and coworkers (174, 175, 260) that caused an altered PTS-mediated inducer exclusion in the mutant strains. In contrast to the original *crr* mutants of *Salmonella typhimurium* and *E. coli*, lacking III^{Glc}, the *iex* mutants still showed a high rate of uptake of methyl α-glucoside and glucose via II^{Glc}. They had normal adenylate cyclase activity, but glucose was unable to exclude lactose and other non-PTS carbohydrates. From this behavior the authors concluded that two distinct regulatory proteins must be involved in the processes described before: (i) III^{Glc}, responsible for the uptake via II^{Glc} as well as for high adenylate cyclase activity; and (ii) a new protein, the product of the *iex* gene, responsible for the regulation of several non-PTS transport systems by inducer exclusion.

Originally, *iex* mutations were claimed to map at the opposite side of *pts* relative to *crr* (46, 260). The iex^+ allele was shown to be dominant over *iex* in complementation tests. After the corresponding DNA fragments were cloned, a single protein of MW 21,000 was identified as the *iex* gene product. All *iex* mutants also contained high levels of III^{Glc} (M_r, 23,000). In subsequent publications by the same authors, it was reported that *iex* does not code for a protein but might be a regulatory gene, controlling expression of the *pts* genes, possibly *ptsHp* (44, 170). A biochemical analysis of authentic *iex* mutants and a series of new mutants with an *iex*-like phenotype was then started (246). One of the original *iex* mutants was found to contain normal amounts of III^{Glc} (as measured by antibodies), in contrast to tight, isogenic *crr* mutants that completely lack soluble III^{Glc}. The III^{Glc} in *iex*

strains functioned normally in transport and phosphorylation, the specific activity reaching 60% of that of wild-type cells. This activity, however, was heat labile, suggesting that the *iex* mutation really affected the structural gene *crr* of III^{Glc}. This assumption is strongly supported by the observation that the mutated III^{Glc} from an *iex* strain could not bind to the lactose permease. Complementation of an *iex* mutation by a crr^+ allele restored the phenotype to Iex^+, and the III^{Glc} produced in such a diplogenotic strain from the crr^+ gene was heat stable and bound normally to the lactose permease. According to these data, *iex* is simply a new allele of *crr*, altering the structure of III^{Glc} in such a way that it becomes heat labile and is unable to bind to permeases or to glycerol kinase while retaining a near normal phosphorylation activity for III^{Glc}. The inability of the mutated protein to bind efficiently to carriers is clearly sufficient to explain the resistance of *iex* mutants to inducer exclusion that is caused by PTS carbohydrates, and no additional protein is needed to explain their altered phenotype.

The genetic evidence that *iex* and *crr* lie at opposite sides of the *pts* operon seems to be an argument against the hypothesis that *iex* mutations are a special class of *crr* mutations. The published evidence is not sufficient to localize the *iex* mutation unequivocally at one or the other side of *pts*. This location was deduced from complementation tests, using transducing phage λiex^+ and *ptsI*(ts) *iex* double mutants at high temperature, at which the thermolabile enzyme I and the thermolabile III^{Glc} were inactive. The dominance of an iex^+ allele over *iex* (260), and the existence of the M_r 21,000 protein also tend to support the notion that *iex* mutations affect III^{Glc}.

DISTRIBUTION OF THE PTS

One may ask why such a complex system as the bacterial PTS has evolved instead of several independent systems, each one specialized in transport, phosphorylation, chemoreception, or the regulation of peripheral catabolic pathways. An answer to this question might be found by looking at the distribution of the PTS in bacteria and by asking which systems can substitute in organisms that lack this system. The evidence for the distribution of the PTS has been reviewed recently (81, 302, 313) and is summarized here. (i) Phosphotransferase systems are widely distributed among procaryotic organisms but have not yet been found in eucaryotes. (ii) They are found preferentially among obligate and facultative anaerobes, whereas they seem to be rare among strict aerobes. All PTS-using bacteria appear capable of metabolizing these carbohydrates via glycolysis. (iii) The glucose-PTS was found in all fermentative bacteria which ferment glucose through the Embden-Meyerhof-Parnas pathway, including homofermentative lactic acid bacteria and a number of *Bacillus* spp. that are able to carry out a (limited) 2,3-butanediol fermentation. It was not found among a series of heterofermentative lactic acid bacteria that ferment glucose by the phospho-ketolase pathway, nor in any strict aerobes. A similar distribution was found in the case of the lactose-PTS. (iv) In phototrophic bacteria and a number of strictly aerobic bacteria (88, 300), a fructose-PTS has been found. These organisms appear to metabolize fructose exclusively or in part through the Embden-Meyerhof-Parnas pathway, whereas other carbohydrates are metabolized via the Entner-Doudoroff pathway. This unexpected metabolism might be related to the fact that fructose is phosphorylated in the C-1 position and enters the Embden-Meyerhof-Parnas pathway at the level of fructose 1,6-bisphosphate. In addition, the singular role of the fructose-PTS in enteric bacteria might reflect an early evolutionary divergence from other phosphotransferase systems. II^{Fru} seems to be phosphorylated by its own FPr. (v) In members of the family *Enterobacteriaceae*, simple hexoses, hexosamines, and hexitols are transported through the PTS, whereas di- and trisaccharides are usually taken up through active transport systems.

CONCLUDING REMARKS

We have described a complex bacterial transport system with multiple functions, which occurs in many different bacteria. In all bacteria studied, it provides, after uptake of the carbohydrate, intracellular carbohydrate phosphates as a source of carbon and energy. It is also involved in the taxis of the cells toward these extracellular carbohydrates. Finally, it regulates the expression of a number of genes involved in catabolism and the enzymatic activity of some of the proteins coded for by these genes. The last two processes have been studied most extensively in enteric bacteria.

Recognition of the various PTS carbohydrates during transport and chemotaxis resides in the membrane-bound enzymes II. In enteric bacteria, the cytoplasmic III^{Glc} is central in regulating the expression or activity of non-PTS uptake systems. Although other soluble enzymes III seem to be present, there is no evidence that they play a role in the regulation of adenylate cyclase and non-PTS uptake systems comparable to that of III^{Glc}. The general PTS proteins, enzyme I and HPr, are involved in accepting the phosphoryl group from PEP and transferring it to the different enzymes II and enzymes III, respectively. No satisfactory answer is possible at the moment to the question why such a complex system has evolved that requires up to four phosphoproteins for the uptake of a solute. A comparable non-PTS carbohydrate such as galactose is taken up via an H^+-galactose symport system (the galactose permease, consisting of a single polypeptide) and converted to galactose 1-phosphate by the corresponding kinase. It should be mentioned, however, that some other non-PTS transport systems, such as those for maltose, galactose (the methyl β-galactoside system), and others are much more complex and may consist of up to four or five different proteins located in the outer membrane, in the periplasmic space, or in the cytoplasmic membrane. It may be significant that these more complicated systems also have a second function, in chemotaxis. One of the components, the periplasmic binding protein, recognizes its substrate and interacts with a membrane-bound component of the chemotactic machinery. None of these systems seems to be as intimately involved in regulation of metabolism, however, as does the PTS. With respect to the large number of proteins involved, it might be very difficult for a protein to evolve that is capable of accepting a phosphoryl group from PEP, reacting with all enzymes II and III, and interacting either in phosphorylated or nonphosphorylated form with a number of membrane-bound and cytoplasmic proteins.

It should be noted that the regulatory system, with III^{Glc} in a central role, has till now only been described in members of the family *Enterobacteriaceae*. This may be due in part to the widespread attention given to this group of organisms. Two recent findings suggest that similar regulatory processes may operate in gram-positive organisms: first, covalent modification of HPr—phosphorylation of a serine residue, catalyzed by a kinase and ATP, modulates HPr activity; second, inducer exclusion of the non-PTS compound glycerol in *B. subtilis* by PTS sugars. It would be interesting to

find proteins similar to III^{Glc} that are also capable of interacting with and modulating several non-PTS proteins in other non-enteric bacteria.

Although complex regulatory phenomena such as catabolite repression, transient repression, and inducer exclusion have been studied extensively, no simple answer can be given as to the molecular mechanisms underlying these processes. It should be clear from this review, however, that PTS-mediated regulation via III^{Glc} plays a major role. It can explain many observations on diauxic growth and the preference of an organism for certain substrates above others. By no means can it explain all of the observations. Future work should be directed to an understanding of these regulatory aspects.

ACKNOWLEDGMENTS

We thank G. Pensel for drawing the figures.

The research in the laboratories of the authors was supported by grants from the Deutsche Forschungsgemeinschaft through SFB4 Teilprojekt C2 and SFB 171-84 Teilprojekte C3 and C4 (to J.W.L.) and the Netherlands Organization for the Advancement of Pure Research (to P.W.P.)

LITERATURE CITED

1. **Adhya, S., and H. Echols.** 1966. Glucose effect and the galactose enzymes of *Escherichia coli*: correlation between glucose inhibition of induction and inducer transport. J. Bacteriol. **92**:601–608.
2. **Adler, J., and W. Epstein.** 1974. Phosphotransferase-system enzymes as chemoreceptors for certain sugars in *Escherichia coli* chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **71**:2895–2899.
3. **Adler, J., G. L. Hazelbauer, and M. M. Dahl.** 1973. Chemotaxis toward sugars in *Escherichia coli*. J. Bacteriol. **115**:824–847.
4. **Ahmed, A., and D. Seraba.** 1975. The nature of the *gal3* mutation of *Escherichia coli*. Mol. Gen. Genet. **136**:233–242.
5. **Aiba, H., K. Mori, M. Tanaka, T. Ooi, A. Roy, and A. Danchin.** 1984. The complete nucleotide sequence of the adenylate cyclase gene of *Escherichia coli*. Nucleic Acids Res. **12**:9427–9440.
6. **Alaeddinoglu, N. G., and H. P. Charles.** 1979. Transfer of a gene for sucrose utilization into *Escherichia coli* K12, and subsequent failure of expression of genes for D-serine utilization. J. Gen. Microbiol. **110**:47–59.
7. **Alexander, J. K.** 1980. Suppression of defects in cyclic adenosine 3′,5′-monophosphate metabolism in *Escherichia coli*. J. Bacteriol. **144**:205–209.
8. **Alexander, J. K., and B. Tyler.** 1975. Genetic analysis of succinate utilization in Enzyme I mutants of the phosphoenolpyruvate:sugar phosphotransferase system in *Escherichia coli*. J. Bacteriol. **124**:252–261.
9. **Alper, M. D., and B. N. Ames.** 1978. Transport of antibiotics and metabolite analogs by systems under cyclic AMP control: positive selection of *Salmonella typhimurium cya* and *crp* mutants. J. Bacteriol. **133**:149–157.
10. **Alpert, C.-A., R. Frank, K. Stüber, J. Deutscher, and W. Hengstenberg.** 1985. The PEP-dependent protein kinase Enzyme I of *Streptococcus faecalis*: purification and the properties of the enzyme and characterization of its active center. Biochemistry **24**:959–964.
11. **Amaral, D., and H. L. Kornberg.** 1975. Regulation of fructose uptake by glucose in *Escherichia coli*. J. Gen. Microbiol. **90**:157–168.
12. **Ammer, J., M. Brennenstuhl, P. Schindler, J. V. Höltje, and H. Zähner.** 1979. Phosphorylation of streptozotocin during uptake via the phosphoenolpyruvate:sugar phosphotransferase system in *Escherichia coli*. Antimicrob. Agents Chemother. **16**:801–807.
13. **Anderson, B., N. Weigel, W. Kundig, and S. Roseman.** 1971. Sugar transport. III. Purification and properties of a phosphocarrier protein (HPr) of the phosphoenolpyruvate-dependent phosphotransferase system of *Escherichia coli*. J. Biol. Chem. **246**:7023–7033.
14. **Anderson, D. G., and L. L. McKay.** 1977. Plasmids, loss of lactose metabolism, and appearance of partial and full lactose-fermenting revertants in *Streptococcus cremoris* B_1. J. Bacteriol. **129**:367–377.
15. **Andrews, K. J., and E. C. C. Lin.** 1976. Selective advantages of various bacterial carbohydrate transport mechanisms. Fed. Proc. **35**:2185–2189.
16. **Arai, T.** 1981. Effect of arsenate on chemotactic behavior of *Escherichia coli*. J. Bacteriol. **145**:803–807.
17. **Asensio, C., G. Avigad, and B. L. Horecker.** 1963. Preferential galactose utilization in a mutant strain of *Escherichia coli*. Arch. Biochem. Biophys. **103**:299–309.
18. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
19. **Bankaitis, V. A., and P. J. Bassford, Jr.** 1982. Regulation of adenylate cyclase synthesis in *Escherichia coli*: studies with *cya-lac* operon and protein fusion strains. J. Bacteriol. **151**:1346–1357.
20. **Begley, G. S., D. E. Hansen, G. R. Jacobson, and J. R. Knowles.** 1982. Stereochemical course of the reactions catalyzed by the bacterial phosphoenolpyruvate:glucose phosphotransferase system. Biochemistry **21**:5552–5556.
21. **Beneski, D. A., T. P. Misko, and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Preparation and characterization of membrane vesicles from mutant and wild type *Salmonella typhimurium*. J. Biol. Chem. **257**:14565–14575.
22. **Berkowitz, D.** 1971. D-Mannitol utilization in *Salmonella typhimurium*. J. Bacteriol. **105**:232–240.
23. **Berman, M., and E. C. C. Lin.** 1971. Glycerol-specific revertants of a phosphoenolpyruvate phosphotransferase mutant: suppression by the desensitization of glycerol kinase to feedback inhibition. J. Bacteriol. **105**:113–120.
24. **Berman, M., N. Zwaig, and E. C. C. Lin.** 1970. Suppression of a pleiotropic mutant affecting glycerol dissimilation. Biochem. Biophys. Res. Commun. **38**:272–278.
25. **Berman-Kurtz, M., E. C. C. Lin, and D. P. Richey.** 1971. Promoter-like mutant with increased expression of the glycerol kinase operon of *Escherichia coli*. J. Bacteriol. **106**:724–731.
26. **Bernsmann, P., C. A. Alpert, P. Muss, J. Deutscher, and W. Hengstenberg.** 1982. The bacterial PEP-dependent phosphotransferase system mechanism of gluconate phosporylation in *Streptococcus faecalis*. FEBS Lett. **138**:102–103.
27. **Beyreuther, K., H. Raufuss, O. Schrecker, and W. Hengstenberg.** 1977. The phosphoenolpyruvate-dependent phosphotransferase system of *Staphylococcus aureus*. 1. Amino-acid sequence of the phosphocarrier protein HPr. Eur. J. Biochem. **75**:275–286.
28. **Bhumiratana, A., R. L. Anderson, and R. N. Costilow.** 1974. Trehalose metabolism by *Bacillus popilliae*. J. Bacteriol. **119**:484–493.
29. **Bisset, D. L., and R. L. Anderson.** 1974. Genetic evidence for the physiological significance of the D-tagatose 6-phosphate pathway of lactose and D-galactose degradation in *Staphylococcus aureus*. J. Bacteriol. **119**:698–704.
30. **Bisset, D. L., W. C. Wener, and R. L. Anderson.** 1980. Lactose and galactose metabolism in *Staphylococcus aureus*. J. Biol. Chem. **255**:8740–8744.
31. **Bitoun, R., H. de Reuse, D. Touati-Schwartz, and A. Danchin.** 1983. The phosphoenolpyruvate dependent carbohydrate phosphotransferase system of *Escherichia coli*. Cloning of the *ptsHI-crr* region and studies with a *pts-lac* operon fusion. FEMS Microbiol. Lett. **16**:163–167.
32. **Black, R. A., A. C. Hobson, and J. Adler.** 1980. Involvement of cyclic GMP in intracellular signalling in the chemotactic response of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:3879–3883.
33. **Black, R. A., A. C. Hobson, and J. Adler.** 1983. Adenylate cyclase is required for chemotaxis to phosphotransferase system sugars by *Escherichia coli*. J. Bacteriol. **153**:1187–1195.

34. **Blumenberg, M., and C. Yanofsky.** 1982. Regulatory region of the *Klebsiella aerogenes* tryptophan operon. J. Bacteriol. **152**:49–56.
35. **Bolshakova, T. N., O. Y. Dobrynina, and V. N. Gershanovitch.** 1979. Isolation and investigation of the *Escherichia coli* mutant with the deletion in the *ptsH* gene. FEBS Lett. **107**:169–172.
36. **Bolshakova, T. N., T. R. Gabrielyan, G. I. Bourd, and V. N. Gershanovitch.** 1978. Involvement of the *Escherichia coli* phosphoenolpyruvate-dependent phosphotransferase system in regulation of transcription of catabolic enzymes. Eur. J. Biochem. **89**:483–490.
37. **Boronat, A., M. C. Jones-Mortimer, and H. L. Kornberg.** 1982. A specialized transducing phage, λ*psrIA*, for the sorbitol phosphotransferase of *Escherichia coli* K-12. J. Gen. Microbiol. **128**:605–611.
38. **Botsford, J. L.** 1981. Cyclic nucleotides in procaryotes. Microbiol. Rev. **45**:620–642.
39. **Botsford, J. L.** 1984. Cyclic AMP phosphodiesterase in *Salmonella typhimurium*: characteristics and physiological function. J. Bacteriol. **160**:826–830.
40. **Botsford, J. L., and M. Drexler.** 1978. The cyclic 3′,5′-adenosine monophosphate receptor protein and regulation of cyclic 3′,5′-adenosine monophosphate synthesis in *Escherichia coli*. Mol. Gen. Genet. **165**:47–56.
41. **Bourd, G. I., R. S. Erlagaeva, T. N. Bolshakova, and V. N. Gershanovitch.** 1975. Glucose catabolite repression in *Escherichia coli* K12 mutants defective in methyl-α-D-glucoside transport. Eur. J. Biochem. **53**:419–427.
42. **Boyd, A., and M. I. Simon.** 1982. Bacterial chemotaxis. Annu. Rev. Physiol. **44**:501–517.
43. **Brickman, E., L. Soll, and J. Beckwith.** 1973. Genetic characterization of mutations which effect catabolite-sensitive operons in *Escherichia coli*, including deletions of the gene for adenyl cyclase. J. Bacteriol. **116**:582–587.
44. **Britton, P., A. Boronat, D. A. Hartley, M. C. Jones-Mortimer, H. L. Kornberg, and F. Parra.** 1983. Phosphotransferase-mediated regulation of carbohydrate utilization in *Escherichia coli* K12: location of the *gsr* (*tgs*) and *iex* (*crr*) genes by specialized transduction. J. Gen. Microbiol. **129**:349–358.
45. **Britton, P., L. G. Lee, D. Murfitt, A. Boronat, M. C. Jones-Mortimer, and H. L. Kornberg.** 1984. Location and direction of transcription of the *ptsH* and *ptsI* genes on the *Escherichia coli* K12 genome. J. Gen. Microbiol. **130**:861–868.
46. **Britton, P., D. Murfitt, M. C. Jones-Mortimer, and H. L. Kornberg.** 1982. Phosphotransferase-mediated regulation of carbohydrate utilization in *Escherichia coli* K12: identification of the products of genes on specialised transducing phages λ*iex* (*crr*) and λ*gsr* (*tgs*). EMBO J. **1**:907–911.
47. **Brouwer, M., M. G. L. Elferink, and G. T. Robillard.** 1982. Phosphoenolypyruvate-dependent fructose phosphotransferase system of *Rhodopseudomonas sphaeroides*: purification and physicochemical and immuno-chemical characterization of a membrane-associated Enzyme I. Biochemistry **21**:82–88.
48. **Button, D. K., J. B. Egan, W. Hengstenberg, and M. L. Morse.** 1973. Carbohydrate transport in *Staphylococcus aureus*. IV. Maltose accumulation and metabolism. Biochem. Biophys. Res. Commun. **52**:880–885.
49. **Calmes, R.** 1978. Involvement of phosphoenolpyruvate in catabolism of caries-conductive disaccharides by *Streptococcus mutans*: lactose transport. Infect. Immun. **19**:934–942.
50. **Castro, L., B. U. Feucht, M. L. Morse, and M. H. Saier, Jr.** 1976. Regulation of carbohydrate permeases and adenylate cyclase in *Escherichia coli*. Studies with mutant strains in which Enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system is thermolabile. J. Biol. Chem. **251**: 5522–5527.
51. **Chalumeau, H., A. Delobbe, and P. Gay.** 1978. Biochemical and genetic study of D-glucitol transport and catabolism in *Bacillus subtilis*. J. Bacteriol. **134**:920–928.
52. **Chassy, B. M., and J. Thompson.** 1983. Regulation of lactose-phosphoenolpyruvate-dependent phosphotransferase system and β-D-phosphogalactoside galactohydrolase activities in *Lactobacillus casei*. J. Bacteriol. **154**:1195–1203.
53. **Chassy, B. M., and J. Thompson.** 1983. Regulation and characterization of the galactose-phosphoenolpyruvate-dependent phosphotransferase system in *Lactobacillus casei*. J. Bacteriol. **154**:1204–1214.
54. **Clark, B., and W. H. Holmes.** 1976. Control of the sequential utilization of glucose and fructose by *Escherichia coli*. J. Gen. Microbiol. **95**:191–201.
55. **Clark, D. J., and A. G. Marr.** 1964. Studies on the repression of β-galactosidase in *Escherichia coli*. Biochim. Biophys. Acta **92**:85–91.
56. **Cohn, M., and K. Horibata.** 1959. Inhibition by glucose of the induced synthesis of the β-galactoside-enzyme system of *Escherichia coli*. Analysis of maintenance. J. Bacteriol. **78**:601–612.
57. **Cohn, M., and K. Horibata.** 1959. Analysis of the differentiation and of the heterogeneity within a population of *Escherichia coli* undergoing induced β-galactosidase synthesis. J. Bacteriol. **78**:613–623.
58. **Cohn, M., and K. Horibata.** 1959. Physiology of the inhibition by glucose of the induced synthesis of the β-galactoside-enzyme system of *Escherichia coli*. J. Bacteriol. **78**:624–635.
59. **Cordaro, J. C.** 1976. Genetics of the bacterial phosphoenolpyruvate:glycose phosphotransferase system. Annu. Rev. Genet. **10**:341–359.
60. **Cordaro, J. C., R. P. Anderson, E. W. Grogan, Jr., D. J. Wenzel, M. Engler, and S. Roseman.** 1974. Promoter-like mutation affecting HPr and enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system in *Salmonella typhimurium*. J. Bacteriol. **120**:245–252.
61. **Cordaro, J. C., T. Melton, J. P. Stratis, M. Atagün, C. Gladding, P. E. Hartman, and S. Roseman.** 1976. Fosfomycin resistance: selection method for internal and extended deletions of the phosphoenolpyruvate:sugar phosphotransferase genes of *Salmonella typhimurium*. J. Bacteriol. **128**:785–793.
62. **Cordaro, J. C., and S. Roseman.** 1972. Deletion mapping of the genes coding for HPr and enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system in *Salmonella typhimurium*. J. Bacteriol. **112**:17–29.
63. **Crenon, I., and A. Ullmann.** 1984. The role of cyclic AMP excretion in the regulation of enzyme synthesis in *Escherichia coli*. FEMS Microbiol. Lett. **22**:47–51.
64. **Csonka, L. N., and A. J. Clark.** 1979. Deletions generated by the transposon Tn10 in the *srl recA* region of the *Escherichia coli* K12 chromosome. Genetics **93**:321–343.
65. **Curtis, S. J., and W. Epstein.** 1975. Phosphorylation of D-glucose in *Escherichia coli* mutants defective in glucosephosphotransferase, mannosephosphotransferase, and glucokinase. J. Bacteriol. **122**:1189–1199.
66. **Dahl, R., H. G. Morse, and M. L. Morse.** 1974. Carbohydrate transport and cyclic 3′,5′ adenosine monophosphate (cAMP) levels in a temperature sensitive phosphotransferase mutant of *Escherichia coli*. Mol. Gen. Genet. **129**:1–10.
67. **Danchin, A., L. Dondon, and J. Daniel.** 1984. Metabolic alterations mediated by 2-keto-butyrate in *Escherichia coli* K12. Mol. Gen. Genet. **193**:473–478.
68. **Danchin, A., N. Guiso, A. Roy, and A. Ullmann.** 1984. Identification of the *Escherichia coli cya* gene product as authentic adenylate cyclase. J. Mol. Biol. **175**:403–408.
69. **Daniel, J.** 1984. Enzyme III stimulation of cyclic AMP synthesis in an *Escherichia coli crp* mutant. J. Bacteriol. **157**:940–941.
70. **Daniel, J., E. Joseph, and A. Danchin.** 1984. Role of 2-keto-butyrate as an alarmone in *Escherichia coli* K-12: inhibition of adenylate cyclase activity mediated by the phosphoenolpyruvate: glycose phosphotransferase transport system. Mol. Gen. Genet. **193**:467–472.
71. **Defez, R., and M. De Felice.** 1982. The metabolism of β-glucosides in *Escherichia coli* K-12. Ann. Microbiol. **133A**: 347–350.
72. **Delidakis, C. E., and M. C. Jones-Mortimer, and H. L. Kornberg.** 1982. A mutant inducible for galactitol utilization in *Escherichia coli* K-12. J. Gen. Microbiol. **128**:601–604.
73. **Delobbe, A., H. Chalumeau, J. M. Claverie, and P. Gay.** 1976. Phosphorylation of intracellular fructose in *Bacillus subtilis* mediated by phosphoenolpyruvate-1-fructose phosphotrans-

ferase. Eur. J. Biochem. **66**:485–491.
74. **Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman.** 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61–76.
75. **De Reuse, H., E. Huttner, and A. Danchin.** 1984. Analysis of the *ptsH-ptsI-crr* region in *Escherichia coli* K-12: evidence for the existence of a single transcription unit. Gene **32**:31–40.
75a. **De Reuse, H., A. Roy, and A. Danchin.** 1985. Analysis of the *ptsH-ptsI-crr* region in *Escherichia coli* K-12: nucleotide sequence of the *ptsH* gene. Gene **35**:199–207.
76. **Deutscher, J., K. Beyreuther, H. M. Sobek, K. Stüber, and W. Hengstenberg.** 1982. Phosphoenolpyruvate-dependent phosphotransferase system of *Staphylococcus aureus*: Factor III^{Lac}, a trimeric phospho carrier protein that also acts as a phase transfer catalyst. Biochemistry **21**:4867–4873.
77. **Deutscher, J., and R. Engelmann.** 1984. Purification and characterization of an ATP-dependent protein kinase from *Streptococcus faecalis*. FEMS Microbiol. Lett. **23**:157–162.
78. **Deutscher, J., U. Kessler, C. A. Alpert, and W. Hengstenberg.** 1984. The bacterial phosphoenolpyruvate dependent phosphotransferase system: P-ser-HPr and its possible regulatory function. Biochemistry **23**:4455–4460.
79. **Deutscher, J., and M. H. Saier, Jr.** 1983. ATP-dependent protein kinase-catalyzed phosphorylation of a seryl residue in HPr, a phosphate carrier protein of the phosphotransferase system in *Streptococcus pyogenes*. Proc. Natl. Acad. Sci. U.S.A. **80**:6790–6794.
80. **Dietz, G. W., Jr.** 1976. The hexose phosphate transport system of *Escherichia coli*. Adv. Enzymol. **44**:237–259.
81. **Dills, S. S., A. Apperson, M. R. Schmidt, and M. H. Saier, Jr.** 1980. Carbohydrate transport in bacteria. Microbiol. Rev. **44**:385–418.
82. **Dills, S. S., M. R. Schmidt, and M. H. Saier, Jr.** 1982. Regulation of lactose transport by the phosphoenolpyruvate-sugar phosphotransferase system in membrane vesicles of *Escherichia coli*. J. Cell. Biochem. **18**:239–244.
83. **Dills, S. S., and S. Seno.** 1983. Regulation of hexitol catabolism in *Streptococcus mutans*. J. Bacteriol. **153**:861–866.
84. **Dobrogosz, W. J., G. W. Hall, D. O. Silva, D. Sherba, J. G. Harman, and T. Melton.** 1983. Regulatory interactions among the *cya*, *crp* and *pts* gene products in *Salmonella typhimurium*. Mol. Gen. Genet. **192**:477–486.
85. **Dooijewaard, G., F. F. Roossien, and G. T. Robillard.** 1979. *Escherichia coli* phosphoenolpyruvate-dependent phosphotransferase system. Copurification of HPr and 1-6 glucan. Biochemistry **18**:2990–2996.
86. **Dörschug, M., R. Frank, H. R. Kalbitzer, W. Hengstenberg, and J. Deutscher.** 1984. Phosphoenolpyruvate-dependent phosphorylation site in enzyme III^{Glc} of the *Escherichia coli* phosphotransferase system. Eur. J. Biochem. **144**:113–119.
87. **Doudoroff, M., W. Z. Hassid, E. W. Putnam, A. L. Potter, and J. Lederberg.** 1949. Direct utilization of maltose by *Escherichia coli*. J. Biol. Chem. **179**:921–929.
88. **Durham, D. R., and P. V. Phibbs, Jr.** 1982. Fractionation and characterization of the phosphoenolpyruvate:fructose 1-phosphotransferase system from *Pseudomonas auruginosa*. J. Bacteriol. **149**:534–541.
89. **Egan, J. B., and M. L. Morse.** 1965. Carbohydrate transport in *Staphylococcus aureus*. I. Genetic and biochemical analysis of a pleiotropic transport mutant. Biochim. Biophys. Acta **97**:310–319.
90. **Egan, J. B., and M. L. Morse.** 1965. Carbohydrate transport in *Staphylococcus aureus*. II. Characterization of the defect of a pleiotropic transport mutant. Biochim. Biophys. Acta **109**:172–183.
91. **Egan, J. B., and M. L. Morse.** 1966. Carbohydrate transport in *Staphylococcus aureus*. III. Studies of the transport process. Biochim. Biophys. Acta **112**:63–73.
92. **Elliot, J., and W. Arber.** 1978. *E. coli* K-12 *pel* mutants, which block phage DNA injection, coincide with *ptsM*, which determines a component of a sugar transport system. Mol. Gen. Genet. **161**:1–8.
93. **Elvin, C. M., and H. L. Kornberg.** 1982. A mutant β-D-glucoside transport system of *Escherichia coli* resistant to catabolite inhibition. FEBS Lett. **147**:137–142.
94. **Englesberg, E., J. A. Watson, and P. A. Hoffee.** 1961. The glucose effect and the relationship between glucose permease, acid phosphatase, and glucose resistance. Cold Spring Harbor Symp. Quant. Biol. **26**:261–275.
95. **Epps, H. M. R., and E. F. Gale.** 1942. The influence of the presence of glucose during growth on the enzymic activities of *Escherichia coli*: comparison of the effect with that produced by fermentation acids. Biochem. J. **36**:619–623.
96. **Epstein, W., S. Jewett, and C. F. Fox.** 1970. Isolation and mapping of phosphotransferase mutants in *Escherichia coli*. J. Bacteriol. **104**:793–797.
97. **Epstein, W., L. B. Rothman-Denes, and J. Hesse.** 1975. Adenosine 3′-5′-cyclic monophosphate as mediator of catabolite repression in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:2300–2304.
98. **Erlagaeva, R. S., T. N. Bolshakova, M. V. Shulgina, G. I. Bourd, and V. Gershanovitch.** 1977. Glucose effect in *tgl* mutant of *Escherichia coli* K-12 defective in methyl-α-D-glucoside transport. Eur. J. Biochem. **72**:127–135.
99. **Erni, B., H. Trachsel, P. W. Postma, and J. P. Rosenbusch.** 1982. Bacterial phosphotransferase system. Solubilization and purification of the glucose-specific Enzyme II from membranes of *Salmonella typhimurium*. J. Biol. Chem. **257**:13726–13730.
100. **Ferenci, T., and H. L. Kornberg.** 1973. The utilization of fructose by *Escherichia coli*. Properties of a mutant defective in fructose 1-phosphate kinase activity. Biochem. J. **132**:341–347.
101. **Ferenci, T., and H. L. Kornberg.** 1974. The role of phosphotransferase synthesis of fructose 1-phosphate and fructose 6-phosphate in the growth of *Escherichia coli* on fructose. Proc. R. Soc. London, Ser. B **187**:105–119.
102. **Feucht, B. U., and M. H. Saier, Jr.** 1980. Fine control of adenylate cyclase by the phosphoenolpyruvate:sugar phosphotranferase systems in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **141**:603–610.
103. **Fox, C. F., and G. Wilson.** 1968. The role of a phosphoenolpyruvate dependent kinase system in β-glucoside catabolism in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **59**:988–995.
104. **Fraenkel, D. G.** 1968. The accumulation of glucose 6-phosphate from glucose and its effect in an *Escherichia coli* mutant lacking phosphoglucose isomerase and glucose 6-phosphate dehydrogenase. J. Biol. Chem. **243**:6451–6457.
105. **Fraenkel, D. G.** 1968. The phosphoenolpyruvate-initiated pathway of fructose metabolism in *Escherichia coli*. J. Biol. Chem. **243**:6458–6463.
106. **Friedman, S. A., and J. B. Hays.** 1977. Initial characterization of hexose and hexitol phosphoenolpyruvate-dependent phosphotransferase of *Staphylococcus aureus*. J. Bacteriol. **130**:991–999.
107. **Futai, M.** 1978. Experimental systems for the study of active transport in bacteria, p. 7–41. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, Inc., New York.
108. **Gachelin, G.** 1969. A new assay of the phosphotransferase system in *Escherichia coli*. Biochem. Biophys. Res. Commun. **34**:382–387.
109. **Gachelin, G.** 1970. Studies on the α-methylglucoside permease of *Escherichia coli*. A two-step mechanism for the accumulation of α-methylglucoside 6-phosphate. Eur. J. Biochem. **16**:342–357.
110. **Galloway, R. J., and B. L. Taylor.** 1980. Histidine starvation and adenosine 5′-triphosphate depletion in chemotaxis of *Salmonella typhimurium*. J. Bacteriol. **144**:1068–1075.
111. **Gauthier, L., D. Mayrand, and C. Vadeboncoeur.** 1984. Isolation of a novel protein involved in the transport of fructose by an inducible phosphoenolpyruvate fructose phosphotransferase system in *Streptococcus mutans*. J. Bacteriol. **160**: 755–763.
112. **Gay, P., P. Cordier, M. Marquet, and A. Delobbe.** 1973. Carbohydrate metabolism and transport in *Bacillus subtilis*. A study of *ctr* mutations. Mol. Gen. Genet. **121**:355–368.

113. **Gee, D. L., P. Baumann, and L. Baumann.** 1975. Enzymes of D-fructose catabolism in species of *Beneckea* and *Photobacterium*. Arch. Microbiol. **103**:205–207.
114. **Gershanovitch, V. N., G. Bourd, N. V. Jurovitzkaya, A. G. Skavronskaya, V. V. Klyutchova, and V. P. Shabolenko.** 1967. β-Galactosidase induction in cells of *Escherichia coli* not utilizing glucose. Biochim. Biophys. Acta **134**:188–190.
115. **Gershanovitch, V. N., T. S. Ilyina, O. Y. Rusina, N. V. Yourovitskaya, and T. N. Bolshakova.** 1977. Repression of inducible enzyme synthesis in a mutant of *Escherichia coli* K-12 deleted for the *ptsH* gene. Mol. Gen. Genet. **153**:185–190.
116. **Glesyna, M. L., T. N. Bolshakova, and V. N. Gershanovitch.** 1983. Effect of *ptsI* and *ptsH* mutations on initiation of transcription of the *Escherichia coli* lactose operon. Mol. Gen. Genet. **190**:417–420.
117. **Goldenbaum, P. E., and G. A. Hall.** 1979. Transport of cyclic adenosine 3',5'-monophosphate across *Escherichia coli* vesicle membranes. J. Bacteriol. **140**:459–467.
118. **Grenier, F. C., E. B. Waygood, and M. H. Saier, Jr.** 1985. Bacterial phosphotransferase system: regulation of mannitol Enzyme II activity by sulfhydryl oxidation. Biochemistry **24**:47–51.
119. **Guidi-Rontani, C., A. Danchin, and A. Ullmann.** 1980. Catabolite repression in *Escherichia coli* mutants lacking cyclic AMP receptor protein. Proc. Natl. Acad. Sci. U.S.A. **77**:5799–5801.
120. **Guidi-Rontani, C., A. Danchin, and A. Ullmann.** 1981. Isolation and characterization of an *Escherichia coli* mutant affected in the regulation of adenylate cyclase. J. Bacteriol. **148**:753–761.
121. **Guidi-Rontani, C., and B. Gicquel-Sanzey.** 1981. Expression of the maltose regulon in strains lacking the cyclic AMP receptor protein. FEMS Microbiol. Lett. **10**:383–387.
122. **Guiso, N., and B. Blazy.** 1980. Regulatory aspects of the cyclic AMP receptor protein in *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. **94**:278–283.
123. **Hagihara, H., T. H. Wilson, and E. C. C. Lin.** 1963. Studies on the glucose-transport system in *Escherichia coli* with α-methylglucoside as substrate. Biochim. Biophys. Acta **78**:505–515.
124. **Haguenauer, R., and A. Kepes.** 1971. The cycle of renewal of intracellular α-methyl glucoside accumulation by the glucose permease of *E. coli*. Biochimie **53**:99–107.
125. **Haguenauer, R., and A. Kepes.** 1972. Sodium fluoride inhibition of phosphorylation and dephosphorylation involved in alpha-methyl-D glucoside transport in *Escherichia coli* K-12. A pH dependent phenomenon sensitive to uncoupling agents. Biochimie **54**:505–512.
126. **Haguenauer-Tsapis, R., and A. Kepes.** 1980. Different sidedness of functionally homologous essential thiols in two membrane-bound phosphotransferase enzymes of *Escherichia coli* detected by permeant and non-permeant thiol reagents. J. Biol. Chem. **255**:5075–5081.
127. **Hall, B. G., K. Imai, and C. P. Romano.** 1982. Genetics of the lac-PTS system of *Klebsiella*. Genet. Res. **39**:287–302.
128. **Hanson, T. E., and R. L. Anderson.** 1968. Phosphoenolpyruvate-dependent formation of D-fructose 1-phosphate by a four component phosphotransferase system. Proc. Natl. Acad. Sci. U.S.A. **61**:269–276.
129. **Harman, J. G., and W. J. Dobrogosz.** 1983. Mechanism of CRP-mediated *cya* suppression in *Escherichia coli*. J. Bacteriol. **153**:191–199.
130. **Harwood, J. P., C. Gazdar, C. Prasad, A. Peterkofsky, S. J. Curtis, and W. Epstein.** 1976. Involvement of the glucose Enzymes II of the sugar phosphotransferase system in the regulation of adenylate cyclase by glucose in *Escherichia coli*. J. Biol. Chem. **251**:2462–2468.
131. **Harwood, J. P., and A. Peterkofsky.** 1975. Glucose-sensitive adenylate cyclase in toluene-treated cells of *Escherichia coli* B. J. Biol. Chem. **250**:4656–4662.
132. **Hays, J. B.** 1978. Group translocation transport systems, p. 43–102. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, Inc., New York.
133. **Hays, J. B., R. D. Simoni, and S. Roseman.** 1973. Sugar transport. V. A trimeric lactose-specific phosphocarrier protein of the *Staphylococcus aureus* phosphotransferase system. J. Biol. Chem. **248**:941–956.
134. **Hazelbauer, G. L., and S. Harayama.** 1983. Sensory transduction in bacterial chemotaxis. Int. Rev. Cytol. **81**:33–70.
135. **Hengge, R., and W. Boos.** 1983. Maltose and lactose transport in *Escherichia coli*. Examples of two different types of concentrative transport systems. Biochim. Biophys. Acta **737**: 443–478.
136. **Hengstenberg, W.** 1977. Enzymology of carbohydrate transport in bacteria. Curr. Top. Microbiol. Immunol. **77**:97–126.
137. **Hernandez-Asensio, M., and F. F. Del Campo.** 1980. Enhancement of α-methylglucoside efflux by respiration in respiratory mutants of *Escherichia coli* K-12. Arch. Biochem. Biophys. **200**:309–318.
138. **Hoffee, P., and E. Englesberg.** 1962. Effect of metabolic activity on the glucose permease of bacterial cells. Proc. Natl. Acad. Sci. U.S.A. **48**:1759–1765.
139. **Hoffee, P., E. Englesberg, and F. Lamy.** 1964. The glucose permease system in bacteria. Biochim. Biophys. Acta **79**: 337–350.
140. **Hommes, R. W. J., P. W. Postma, O. M. Neijssel, D. W. Tempest, P. Dokter, and J. A. Duine.** 1984. Evidence of a quinoprotein glucose dehydrogenase apoenzyme in several strains of *Escherichia coli*. FEMS Microbiol. Lett. **24**:329–333.
141. **Hori, H., and S. Osawa.** 1978. Evolution of ribosomal proteins in *Enterobacteriaceae*. J. Bacteriol. **133**:1089–1095.
142. **Hoving, H., R. ten Hoeve-Duurkens, and G. T. Robillard.** 1984. *Escherichia coli* phosphoenolpyruvate-dependent phosphotransferase system. Functional asymmetry in *Escherichia coli* Enzyme I subunits demonstrated by reaction with 3-bromopyruvate. Biochemistry **23**:4335–4340.
143. **Huedig, H., and W. Hengstenberg.** 1980. The bacterial phosphoenolpyruvate dependent phosphotransferase system (PTS). Solubilisation and kinetic parameters of the glucose-specific membrane-bound Enzyme II component of *Streptococcus faecalis*. FEBS Lett. **114**:103–106.
144. **Jablonski, E. G., L. Brand, and S. Roseman.** 1983. Sugar transport by the bacterial phosphotransferase system. Preparation of a fluorescein derivative of the glucose-specific phosphocarrier protein III^{Glc} and its binding to the phosphocarrier protein HPr. J. Biol. Chem. **258**:9690–9699.
145. **Jacobson, G. R., D. M. Kelly, and D. R. Finlay.** 1983. The intramembrane topography of the mannitol-specific Enzyme II of the *Escherichia coli* phosphotransferase system. J. Biol. Chem. **258**:2955–2959.
146. **Jacobson, G. R., C. A. Lee, J. E. Leonard, and M. H. Saier, Jr.** 1983. Mannitol-specific Enzyme II of the bacterial phosphotransferase system. I. Properties of the purified permease. J. Biol. Chem. **258**:10748–10756.
147. **Jacobson, G. R., C. A. Lee, and M. H. Saier, Jr.** 1979. Purification of the mannitol-specific Enzyme II of the *Escherichia coli* phosphoenolpyruvate: sugar phosphotransferase system. J. Biol. Chem. **254**:249–252.
148. **Jacobson, G. R., L. E. Tanney, D. M. Kelly, K. B. Palman, and S. B. Corn.** 1983. Substrate and phospholipid specificity of the purified mannitol permease of *Escherichia coli*. J. Cell. Biochem. **23**:231–240.
149. **Jaffor Ullah, A. H., and V. P. Cirillo.** 1976. *Mycoplasma* phosphoenolpyruvate-dependent sugar phosphotransferase system: purification and characterization of the phosphocarrier protein. J. Bacteriol. **127**:1298–1306.
150. **Jaffor Ullah, A. H., and V. P. Cirillo.** 1977. Mycoplasma phosphoenolpyruvate-dependent sugar phosphotransferase system: purification and characterization of enzyme I. J. Bacteriol. **131**:988–996.
151. **Janecek, J., Z. Naprstek, M. Dobrova, M. Jiresova, and M. Spizek.** 1979. Adenylate cyclase activity in *Escherichia coli* cultured under various conditions. FEMS Microbiol. Lett. **6**:305–307.
152. **Jin, R. Z., and E. C. C. Lin.** 1984. An inducible phosphoenolpyruvate: dihydroxyacetone phosphotransferase system in *Escherichia coli*. J. Gen. Microbiol. **130**:83–88.

153. **Jones-Mortimer, M. C., and H. L. Kornberg.** 1974. Genetical analysis of fructose utilization by *Escherichia coli*. Proc. R. Soc. London Series B **187**:121–131.
154. **Jones-Mortimer, M. C., and H. L. Kornberg.** 1974. Genetic control of inducer exclusion by *Escherichia coli*. FEBS Lett. **48**:93–95.
155. **Jones-Mortimer, M. C., and H. L. Kornberg.** 1976. Uptake of fructose by the sorbitol phosphotransferase of *Escherichia coli* K-12. J. Gen. Microbiol. **96**:383–391.
156. **Jones-Mortimer, M. C., and H. L. Kornberg.** 1980. Amino-sugar transport systems of *Escherichia coli*. J. Gen. Microbiol. **117**:369–376.
157. **Joseph, E., C. Bernsley, N. Guiso, and A. Ullmann.** 1982. Multiple regulation of the activity of adenylate cyclase in *Escherichia coli*. Mol. Gen. Genet. **185**:262–268.
158. **Kaback, H. R.** 1968. The role of the phosphoenolpyruvate-phosphotransferase system in the transport of sugars by isolated membrane preparations of *Escherichia coli*. J. Biol. Chem. **243**:3711–3724.
159. **Kaback, H. R.** 1969. Regulation of sugar transport in isolated bacterial membrane preparations from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **63**:724–731.
160. **Kalbitzer, H. R., W. Hengstenberg, P. Rosch, P. Muss, P. Bernsmann, R. Engelmann, M. Dörschug, and J. Deutscher.** 1982. HPr proteins of different microorganisms studied by hydrogen-1-high-resolution nuclear magnetic resonance: similarities of structures and mechanism. Biochemistry **21**: 2879–2885.
161. **Kelker, N. E., and R. L. Anderson.** 1971. Sorbitol metabolism in *Aerobacter aerogenes*. J. Bacteriol. **105**:160–164.
162. **Kelker, N. E., and R. L. Anderson.** 1972. Evidence for vectorial phosphorylation of D-fructose by intact cells of *Aerobacter aerogenes*. J. Bacteriol. **112**:1441–1443.
163. **Kelker, N. E., R. A. Simkins, and R. L. Anderson.** 1972. Pathway of L-sorbose metabolism in *Aerobacter aerogenes*. J. Biol. Chem. **247**:1479–1483.
164. **Kepes, A.** 1960. Etudes cinétiques sur la galactoside-permease d'*Escherichia coli*. Biochim. Biophys. Acta **40**:70–84.
165. **Klein, W. L., and P. D. Boyer.** 1972. Energization of active transport by *Escherichia coli*. J. Biol. Chem. **247**:7257–7265.
166. **Kolb, A., A. Spassky, C. Chapon, B. Blazy, and H. Buc.** 1983. On the different binding affinities of CRP at the *lac*, *gal*, and *malT* promoter regions. Nucleic Acids Res. **11**:7833–7852.
167. **Konings, W. N., and G. T. Robillard.** 1982. Physical mechanism for regulation of proton solute symport in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **79**:5480–5484.
168. **Koop, A. H., M. Hartley, and S. Bourgeois.** 1984. Analysis of the *cya* locus of *Escherichia coli*. Gene **28**:133–146.
169. **Kornberg, H. L.** 1973. Fine control of sugar uptake by *Escherichia coli*. Symp. Soc. Exp. Biol. **27**:175–193.
170. **Kornberg, H. L., P. Britton, M. C. Jones-Mortimer, and L. G. Lee.** 1984. The role of the phosphoenolpyruvate-phosphotransferase system in inducer exclusion. Biochem. Soc. Trans. **12**:157–159.
171. **Kornberg, H. L., and M. C. Jones-Mortimer.** 1978. The phosphotransferase system as a site of cellular control. Symp. Soc. Gen. Microbiol. **27**:217–240.
172. **Kornberg, H. L., and C. Riordan.** 1976. Uptake of galactose into *Escherichia coli* by facilitated diffusion. J. Gen. Microbiol. **94**:75–89.
173. **Kornberg, H. L., and P. D. Watts.** 1978. Roles of *crr*-gene products in regulating carbohydrate uptake by *Escherichia coli*. FEBS Lett. **89**:329–339.
174. **Kornberg, H. L., and P. D. Watts.** 1979. *tgs* and *crr*: genes involved in catabolite inhibition and inducer exclusion in *Escherichia coli*. FEBS Lett. **104**:313–316.
175. **Kornberg, H. L., P. D. Watts, and K. Brown.** 1980. Mechanisms of "inducer exclusion" by glucose. FEBS Lett. **117**(Suppl.):K28–K36.
176. **Korte, T., and W. Hengstenberg.** 1971. Purification and characterization of the inducible lactose-specific membrane bound component of the staphylococcal phosphopyruvate-dependent phosphotransferase system. Eur. J. Biochem. **23**:295–302.
177. **Koshland, D. E., Jr.** 1981. Biochemistry of sensing and adaptation in a simple bacterial system. Annu. Rev. Biochem. **50**:765–782.
178. **Kubota, Y., S. Iuchi, A. Fujisawa, and S. Tanaka.** 1979. Separation of four components of the phosphoenolpyruvate: glucose phosphotransferase system in *Vibrio parahaemolyticus*. Microbiol. Immunol. **23**:131–146.
179. **Kubota, Y., S. Iuchi, and S. Tanaka.** 1982. Evidence for the existence of a trehalose-specific enzyme II of the phosphoenolpyruvate:sugar phosphotransferase system in *Vibrio parahaemolyticus*. FEMS Microbiol. Lett. **13**:5–7.
180. **Kukuruzinska, M. A., W. F. Harrington, and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Studies on the molecular weight and association of Enzyme I. J. Biol. Chem. **257**:14470–14476.
181. **Kundig, W.** 1974. Molecular interactions in the bacterial phosphoenolpyruvate-phosphotransferase system (PTS). J. Supramol. Struct. **2**:695–714.
182. **Kundig, W., S. Ghosh, and S. Roseman.** 1964. Phosphate bound to histidine in a protein as an intermediate in a novel phosphotransferase system. Proc. Natl. Acad. Sci. U.S.A. **52**:1067–1074.
183. **Kundig, W., and S. Roseman.** 1971. Sugar transport. I. Isolation of a phosphotransferase system from *Escherichia coli*. J. Biol. Chem. **246**:1393–1406.
184. **Kundig, W., and S. Roseman.** 1971. Sugar transport. II. Characterization of constitutive membrane-bound Enzymes II of the *Escherichia coli* phosphotransferase system. J. Biol. Chem. **246**:1407–1418.
185. **LeBlanc, D. J., V. L. Crow, and L. N. Lee.** 1980. Plasmid mediated carbohydrate catabolic enzymes among strains of *Streptococcus lactis*, p. 31–41. *In* C. Stuttard and K. R. Rooze (ed.), Plasmids and transposons—environmental effects and maintenance metabolism. Academic Press, Inc., New York.
186. **Lee, C. A., G. R. Jacobson, and M. H. Saier, Jr.** 1981. Plasmid-directed synthesis of enzymes required for D-mannitol transport and utilization in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:7336–7340.
187. **Lee, C. A., and M. H. Saier, Jr.** 1983. Mannitol-specific Enzyme II of the bacterial phosphotransferase system. III. The nucleotide sequence of the permease gene. J. Biol. Chem. **258**:10761–10767.
188. **Lee, L. G., P. Britton, F. Parra, A. Boronat, and H. L. Kornberg.** 1982. Expression of the *ptsH*$^+$ gene of *Escherichia coli* cloned on plasmid pBR322. A convenient means for obtaining the histidine-containing protein HPr. FEBS Lett. **149**:288–292.
189. **Leib, T. K., and J. A. Gerlt.** 1983. Evidence for a small catalytic domain in the adenylate cyclase from *Salmonella typhimurium*. J. Biol. Chem. **258**:12982–12987.
190. **Lengeler, J.** 1966. Untersuchungen zum Glucose-Effekt bei der Synthese der Galaktose-Enzyme von *Escherichia coli*. Z. Vererbungl. **98**:203–229.
191. **Lengeler, J.** 1975. Mutations affecting transport of the hexitols D-mannitol, D-glucitol, and galactitol in *Escherichia coli* K-12: isolation and mapping. J. Bacteriol. **124**:26–38.
192. **Lengeler, J.** 1975. Nature and properties of hexitol transport systems in *Escherichia coli*. J. Bacteriol. **124**:39–47.
193. **Lengeler, J.** 1977. Analysis of mutations affecting dissimilation of galactitol (dulcitol) in *Escherichia coli* K-12. Mol. Gen. Genet. **152**:83–91.
194. **Lengeler, J.** 1979. Streptozotocin, an antibiotic superior to penicillin in the selection of rare bacterial mutations. FEMS Microbiol. Lett. **5**:417–419.
195. **Lengeler, J.** 1980. Analysis of the physiological effects of the antibiotic streptozotocin on *Escherichia coli* K-12 and other sensitive bacteria. Arch. Microbiol. **128**:196–203.
196. **Lengeler, J.** 1980. Characterization of mutants of *Escherichia coli* K-12, selected by resistance to streptozotocin. Mol. Gen. Genet. **179**:49–54.
197. **Lengeler, J., A. M. Auburger, R. Mayer, and A. Pecher.** 1981. The phosphoenolpyruvate-dependent carbohydrate: phosphotransferase system Enzymes II as chemoreceptors in chemo-

taxis of *Escherichia coli* K-12. Mol. Gen. Genet. **183**:163–170.
198. **Lengeler, J., K. O. Hermann, H. J. Unsöld, and W. Boos.** 1971. The regulation of the β-methylgalactoside transport system and of the galactose binding protein of *Escherichia coli* K-12. Eur. J. Biochem. **19**:457–470.
199. **Lengeler, J., and E. C. C. Lin.** 1972. Reversal of the mannitol-sorbitol diauxie in *Escherichia coli*. J. Bacteriol. **112**:840–848.
200. **Lengeler, J., R. J. Mayer, and K. Schmid.** 1982. Phosphoenolpyruvate-dependent phosphotransferase system enzyme III and plasmid-encoded sucrose transport in *Escherichia coli*. J. Bacteriol. **151**:468–471.
201. **Lengeler, J., and H. Steinberger.** 1978. Analysis of the regulatory mechanisms controlling the synthesis of the hexitol transport systems in *Escherichia coli* K-12. Mol. Gen. Genet. **164**:163–169.
202. **Lengeler, J., and H. Steinberger.** 1978. Analysis of the regulatory mechanisms controlling the activity of the hexitol transport systems in *Escherichia coli* K-12. Mol. Gen. Genet. **167**:75–82.
203. **Leonard, J. E., and M. H. Saier, Jr.** 1981. Genetic dissection of catalytic activities of the *Salmonella typhimurium* mannitol enzyme II. J. Bacteriol. **145**:1106–1109.
204. **Leonard, J. E., and M. H. Saier, Jr.** 1983. Mannitol-specific Enzyme II of the bacterial phosphotransferase system. II. Reconstitution of vectorial transphosphorylation in phospholipid vesicles. J. Biol. Chem. **258**:10757–10760.
205. **Lepesant, J. A., F. Kunst, M. Pascal, J. Kejzlavorá-Lepesant, M. Steinmetz, and R. Dedonder.** 1976. Specific and pleiotropic regulatory mechanisms in the sucrose system of *Bacillus subtilis* 168, p. 58–69. *In* D. Schlessinger (ed.), Microbiology—1976. American Society for Microbiology, Washington, D.C.
206. **Liberman, E. S., and A. S. Bleiweis.** 1984. Role of the phosphoenolpyruvate-dependent glucose phosphotransferase system of *Streptococcus mutans* GS5 in the regulation of lactose uptake. Infect. Immun. **43**:536–542.
207. **Liberman, E. S., and A. S. Bleiweis.** 1984. Transport of glucose and mannose by a common phosphoenolpyruvate-dependent phosphotransferase system in *Streptococcus mutans* GS5. Infect. Immun. **43**:1106–1109.
208. **Lin, E. C. C.** 1970. The genetics of bacterial transport systems. Annu. Rev. Genet. **4**:225–262.
209. **Lin, E. C. C.** 1976. Glycerol dissimilation and its regulation in bacteria. Annu. Rev. Microbiol. **30**:535–578.
210. **Link, C. D., and A. M. Reiner.** 1982. Inverted repeats surround the ribitol-arabitol genes of *E. coli* C. Nature (London) **298**:94–96.
211. **Link, C. D., and A. M. Reiner.** 1983. Genotypic exclusion: a novel relationship between ribitol-arabitol and galactitol genes of *E. coli*. Mol. Gen. Genet. **189**:337–339.
212. **Lis, J. T., and R. Schleif.** 1973. Different cyclic AMP requirements for induction of the arabinose and lactose operons of *Escherichia coli*. J. Mol. Biol. **79**:149–162.
213. **Liu, K. D., and S. Roseman.** 1983. Kinetic characterization and regulation of phosphoenolpyruvate-dependent methyl α-D-glucopyranoside transport by *Salmonella typhimurium* membrane vesicles. Proc. Natl. Sci. Acad. U.S.A. **80**:7142–7145.
214. **London, J., and S. Z. Hausman.** 1983. Purification and characterization of the III^{Xtl} phospho-carrier protein of the phosphoenolpyruvate-dependent xylitol:phosphotransferase found in *Lactobacillus casei* C183. J. Bacteriol. **156**:611–619.
215. **Macchia, V., G. Caputo, E. Mandato, A. Rocino, S. Adhya, and I. Pastan.** 1981. Guanylate cyclase activity in *Escherichia coli* mutants defective in adenylate cyclase. J. Bacteriol. **147**: 931–934.
216. **MacDonald, C., and M. Riley.** 1983. Cloning chromosomal *lac* genes of *Klebsiella pneumoniae*. Gene **24**:341–345.
217. **Magasanik, B.** 1961. Catabolite repression. Cold Spring Harbor Symp. Quant. Biol. **26**:249–256.
218. **Magasanik, B.** 1970. Glucose effects: Inducer exclusion and repression, p. 189–219. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
219. **Majerfeld, I. H., D. Miller, E. Spitz, and H. V. Rickenberg.** 1981. Regulation of the synthesis of adenylate cyclase in *Escherichia coli* by the cAMP-cAMP receptor protein complex. Mol. Gen. Genet. **181**:470–475.
220. **Maloney, P. C., S. V. Ambudkar, J. Thomas, and L. Schiller.** 1984. Phosphate/hexose 6-phosphate antiport in *Streptococcus lactis*. J. Bacteriol. **158**:238–245.
221. **Maréchal, L. R.** 1984. Transport and metabolism of trehalose in *Escherichia coli* and *Salmonella typhimurium*. Mol. Gen. Genet. **187**:70–73.
222. **Markwell, J., G. T. Shimamoto, D. L. Bisset, and R. L. Anderson.** 1976. Pathway of galactitol catabolism in *Klebsiella pneumoniae*. Biochem. Biophys. Res. Commun. **71**:221–227.
223. **Marquet, M., M. C. Creignou, and R. Dedonder.** 1976. The phosphoenolpyruvate: methyl-α-D-glucoside phosphotransferase system in *Baccillus subtilis* Marburg 168: purification and identification of the phosphocarrier protein (HPr). Biochimie **58**:435–441.
224. **Marquet, M., M. C. Creignou, and R. Dedonder.** 1978. The phosphoenolpyruvate: methyl-α-D-glucoside phosphotransferase system in *Bacillus subtilis* Marburg: kinetic studies of enzyme II and evidence for a phosphoryl enzyme II intermediate. Biochimie **60**:1283–1287.
225. **Mattoo, R. L., R. L. Khandelwal, and E. B. Waygood.** 1984. Isoelectrophoretic separation and the detection of soluble proteins containing acid-labile phosphate: use of the phosphoenolpyruvate: sugar phosphotransferase system as a model for N^1-P-histidine- and N^3-P-histidine-containing proteins. Anal. Biochem. **139**:1–16.
226. **Mattoo, R. L., and E. B. Waygood.** 1983. Determination of the levels of HPr and Enzyme I of the phosphoenolpyruvate-sugar phosphotransferase system in *Escherichia coli* and *Salmonella typhimurium*. Can. J. Biochem. Cell Biol. **61**:29–37.
227. **McEntee, K.** 1977. Genetic analysis of the *Escherichia coli* K-12 *srl* region. J. Bacteriol. **132**:904–911.
228. **Meadow, N. D., and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Isolation and characterization of a glucose-specific protein (III^{Glc}) from *Salmonella typhimurium*. J. Biol. Chem. **257**:14526–14537.
229. **Meadow, N. D., J. M. Rosenberg, H. M. Pinkert, and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Evidence that *crr* is the structural gene for the *Salmonella typhimurium* glucose-specific phosphocarrier protein III^{Glc}. J. Biol. Chem. **257**:14538–14542.
230. **Meadow, N. D., D. W. Saffen, R. P. Dottin, and S. Roseman.** 1982. Molecular cloning of the *crr* gene and evidence that it is the structural gene for III^{Glc}, a phosphocarrier protein of the bacterial phosphotransferase system. Proc. Natl. Acad. Sci. U.S.A. **79**:2528–2532.
231. **Melton, T., P. E. Hartman, J. P. Stratis, T. L. Lee, and A. T. Davis.** 1978. Chemotaxis of *Salmonella typhimurium* to amino acids and some sugars. J. Bacteriol. **133**:708–716.
232. **Melton, T., W. Kundig, P. E. Hartman, and N. Meadow.** 1976. 3-Deoxy-3-fluoro-D-glucose-resistant *Salmonella typhimurium* mutants defective in the phosphoenolpyruvate:glycose phosphotransferase system. J. Bacteriol. **128**:794–800.
233. **Mimura, C. S., L. B. Eisenberg, and G. R. Jacobson.** 1984. Resolution of the phosphotransferase enzymes of *Streptococcus mutans*: purification and preliminary characterization of a heat-stable phosphocarrier protein. Infect. Immun. **44**: 708–715.
234. **Misset, O., M. Blaauw, P. W. Postma, and G. T. Robillard.** 1983. Bacterial phosphoenolpyruvate-dependent phosphotransferase system. Mechanisms of the transmembrane sugar translocation and phosphorylation. Biochemistry **22**: 6163–6170.
235. **Misset, O., M. Brouwer, and G. T. Robillard.** 1980. *Escherichia coli* phosphoenolpyruvate-dependent phosphotransferase system. Evidence that the dimer is the active form of Enzyme I. Biochemistry **19**:883–890.
236. **Misset, O., and G. T. Robillard.** 1982. *Escherichia coli* phosphoenolpyruvate-dependent phosphotransferase system: mechanism of phosphoryl-group transfer from phosphoenolpyruvate to HPr. Biochemistry **21**:3136–3142.

237. **Mitchell, P.** 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. Nature (London) **191**:144–148.
238. **Mitchell, P.** 1967. Translocation through natural membranes. Adv. Enzymol. **29**:33–87.
239. **Mitchell, W. J., T. P. Misko, and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Regulation of other transport systems (lactose and melibiose). J. Biol. Chem. **257**:14553–14564.
240. **Monod, J.** 1942. Recherches sur la croissance des cultures bactériennes. Hermann et Cie, Paris.
241. **Morse, H. G., W. K. Penberthy, and M. L. Morse.** 1971. Biochemical characterization of the *ctr* mutants of *Escherichia coli*. J. Bacteriol. **108**:690–694.
242. **Morse, M. L., K. L. Hill, J. B. Egan, and W. Hengstenberg.** 1968. Metabolism of lactose by *Staphylococcus aureus* and its genetic basis. J. Bacteriol. **95**:2270–2274.
243. **Mugharbil, U., and V. P. Cirillo.** 1978. Mycoplasma phosphoenolpyruvate-dependent sugar phosphotransferase system: glucose-negative mutant and regulation of intracellular cyclic AMP. J. Bacteriol. **133**:203–209.
244. **Murphey, W. H., and E. D. Rosenblum.** 1964. Mannitol catabolism by *Staphylococcus aureus*. Arch. Biochem. Biophys. **107**:292–297.
245. **Murphey, W. H., and E. D. Rosenblum.** 1964. Genetic recombination by transduction between mannitol-negative mutants of *Staphylococcus aureus*. Proc. Soc. Exp. Biol. Med. **116**:544–548.
246. **Nelson, S. O., J. Lengeler, and P. W. Postma.** 1984. Role of III^{Glc} of the phosphoenolpyruvate-glucose phosphotransferase system in inducer exclusion in *Escherichia coli*. J. Bacteriol. **160**:360–364.
247. **Nelson, S. O., and P. W. Postma.** 1984. Interactions in vivo between III^{Glc} of the phosphoenolpyruvate: sugar phosphotransferase system and the glycerol and maltose uptake systems of *Salmonella typhimurium*. Eur. J. Biochem. **139**:29–34.
248. **Nelson, S. O., B. J. Scholte, and P. W. Postma.** 1982. Phosphoenolpyruvate:sugar phosphotransferase system-mediated regulation of carbohydrate metabolism in *Salmonella typhimurium*. J. Bacteriol. **150**:604–615.
249. **Nelson, S. O., A. R. J. Schuitema, R. Benne, L. H. T. van der Ploeg, J. J. Plijter, F. Aan, and P. W. Postma.** 1984. Molecular cloning, sequencing and expression of the *crr* gene: the structural gene for III^{Glc} of the bacterial PEP: glucose phosphotransferase system. EMBO J. **3**:1587–1593.
250. **Nelson, S. O., J. K. Wright, and P. W. Postma.** 1983. The mechanism of inducer exclusion. Direct interaction between purified III^{Glc} of the phosphoenolpyruvate: sugar phosphotransferase system and the lactose carrier of *Escherichia coli*. EMBO J. **2**:715–720.
251. **Neuhaus, J. M., and J. K. Wright.** 1983. Chemical modification of the lactose carrier of *Escherichia coli* by plumbagin, phenyl arsinoxide or diethylpyrocarbonate affects the binding of galactoside. Eur. J. Biochem. **137**:615–621.
252. **Neyssel, O. M., D. W. Tempest, P. W. Postma, J. A. Duine, and J. Frank, Jzn.** 1983. Glucose metabolism by K^+-limited *Klebsiella aerogenes*: evidence for the involvement of a quinoprotein glucose dehydrogenase. FEMS Microbiol. Lett. **20**:35–39.
253. **Niaudet, B., P. Gay, and R. Dedonder.** 1975. Identification of the structural gene of the PEP-phosphotransferase Enzyme I in *Bacillus subtilis* Marburg. Mol. Gen. Genet. **136**:337–349.
254. **Nichols, B. P., M. Blumenberg, and C. Yanofsky.** 1981. Comparison of the nucleotide sequence of *trpA* and sequences immediately beyond the *trp* operon of *Klebsiella aerogenes*, *Salmonella typhimurium*, and *Escherichia coli*. Nucleic Acids Res. **9**:1743–1755.
255. **Niwano, M., and B. L. Taylor.** 1982. Novel sensory adaptation mechanism in bacterial chemotaxis to oxygen and phosphotransferase substrates. Proc. Natl. Acad. Sci. U.S.A. **79**:11–15.
256. **Okada, T., K. Ueyama, S. Niiya, H. Kanazawa, M. Futai, and T. Tsuchiya.** 1981. Role of inducer exclusion in preferential utilization of glucose over melibiose in diauxic growth of *Escherichia coli*. J. Bacteriol. **146**:1030–1037.
257. **Osumi, T., and M. H. Saier, Jr.** 1982. Regulation of lactose permease activity by the phosphoenolpyruvate: sugar phosphotransferase system: evidence for direct binding of the glucose-specific Enzyme III to the lactose permease. Proc. Natl. Acad. Sci. U.S.A. **79**:1457–1461.
258. **Paigen, K.** 1966. Phenomenon of transient repression in *Escherichia coli*. J. Bacteriol. **91**:1201–1209.
259. **Paigen, K., and B. Williams.** 1970. Catabolite repression and other control mechanisms in carbohydrate utilization. Adv. Microb. Physiol. **4**:251–324.
260. **Parra, F., M. C. Jones-Mortimer, and H. L. Kornberg.** 1983. Phosphotransferase-mediated regulation of carbohydrate utilization in *Escherichia coli* K12: The nature of the *iex* (*crr*) and *gsr* (*tgs*) mutations. J. Gen. Microbiol. **129**:337–348.
261. **Pastan, I., and S. Adhya.** 1976. Cyclic adenosine 5′-monophosphate in *Escherichia coli*. Bacteriol. Rev. **40**:527–551.
262. **Pastan, I., and R. L. Perlman.** 1969. Repression of β-galactosidase synthesis by glucose in phosphotransferase mutants of *Escherichia coli*. Repression in the absence of glucose phosphorylation. J. Biol. Chem. **244**:5836–5842.
263. **Pecher, A., I. Renner, and J. Lengeler.** 1984. The phosphoenolpyruvate-dependent carbohydrate: phosphotransferase system enzymes II, a new class of chemosensors in bacterial chemotaxis, p. 517–531. *In* H. Sund and C. Veeger (ed.), Mobility and recognition in cell biology. Walter de Gruyter & Co., Berlin.
264. **Peri, K. G., H. L. Kornberg, and E. B. Waygood.** 1984. Evidence for the phosphorylation of Enzyme $II^{Glucose}$ of the phosphoenolpyruvate: sugar phosphotransferase system of *Escherichia coli* and *Salmonella typhimurium*. FEBS Lett. **170**:55–58.
265. **Perret, J., and P. Gay.** 1979. Kinetic study of a phosphoryl exchange reaction between fructose and fructose 1-phosphate catalyzed by the membrane-bound Enzyme II of the phosphoenolpyruvate-fructose 1-phosphotransferase system of *Bacillus subtilis*. Eur. J. Biochem. **102**:237–246.
266. **Peterkofsky, A.** 1981. *Escherichia coli* adenylate cyclase as a sensor of sugar transport function. Adv. Cyclic Nucleotide Res. **14**:215–228.
267. **Peterkofsky, A., and C. Gazdar.** 1973. Measurement of rates of adenosine 3′:5′-cyclic monophosphate synthesis in intact *Escherichia coli* B. Proc. Natl. Acad. Sci. U.S.A. **70**: 2149–2152.
268. **Peterkofsky, A., and C. Gazdar.** 1974. Glucose inhibition of adenylate cyclase in intact cells of *Escherichia coli* B. Proc. Natl. Acad. Sci. U.S.A. **71**:2324–2328.
269. **Peterkofsky, A., and C. Gazdar.** 1975. Interaction of Enzyme I of the phosphoenolpyruvate: sugar phosphotransferase system with adenylate cyclase of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:2920–2924.
270. **Peterkofsky, A., and C. Gazdar.** 1979. *Escherichia coli* adenylate cyclase complex: regulation by the proton electrochemical gradient. Proc. Natl. Acad. Sci. U.S.A. **76**:1099–1103.
271. **Postma, P. W.** 1976. Involvement of the phosphotransferase system in galactose transport in *Salmonella typhimurium*. FEBS Lett. **61**:49–53.
272. **Postma, P. W.** 1977. Galactose transport in *Salmonella typhimurium*. J. Bacteriol. **129**:630–639.
273. **Postma, P. W.** 1981. Defective enzyme $II\text{-}B^{Glucose}$ of the phosphoenolpyruvate:sugar phosphotransferase system leading to uncoupling of transport and phosphorylation in *Salmonella typhimurium*. J. Bacteriol. **147**:382–389.
274. **Postma, P. W.** 1982. Regulation of sugar transport in *Salmonella typhimurium*. Ann. Microbiol. **133A**:261–267.
275. **Postma, P. W., J. C. Cordaro, and S. Roseman.** 1977. Sugar transport. A pleiotropic membrane mutant of *Salmonella typhimurium*. J. Biol. Chem. **252**:7862–7876.
276. **Postma, P. W., W. Epstein, A. R. J. Schuitema, and S. O. Nelson.** 1984. Interaction between III^{Glc} of the phosphoenolpyruvate:sugar phosphotransferase system and glycerol

kinase of *Salmonella typhimurium*. J. Bacteriol. **158**:351–353.
277. **Postma, P. W., and O. M. Neyssel, and R. van Ree.** 1982. Glucose transport in *Salmonella typhimurium* and *Escherichia coli*. Eur. J. Biochem. **123**:113–119.
278. **Postma, P. W., and S. Roseman.** 1976. The bacterial phosphoenolpyruvate:sugar phosphotransferase system. Biochim. Biophys. Acta **457**:213–257.
279. **Postma, P. W., and B. J. Scholte.** 1979. Regulation of sugar transport in *Salmonella typhimurium*, p. 249–257. *In* E. Quagliariello et al. (ed.), Function and molecular aspects of biomembrane transport. Elsevier Biomedical Press, Amsterdam.
280. **Postma, P. W., A. Schuitema, and C. Kwa.** 1981. Regulation of methyl β-galactoside permease activity in *pts* and *crr* mutants of *Salmonella typhimurium*. Mol. Gen. Genet. **181**:448–453.
281. **Postma, P. W., and J. B. Stock.** 1980. Enzymes II of the phosphotransferase system do not catalyze sugar transport in the absence of phosphorylation. J. Bacteriol. **141**:476–484.
282. **Powers, D. A., and S. Roseman.** 1985. The primary structure of *Salmonella typhimurium* HPr, a phosphocarrier protein of the phosphoenolpyruvate: glycose phosphotransferase system. A correction. J. Biol. Chem. **259**:15212–15214.
283. **Prasad, I., and S. Schaefler.** 1974. Regulation of the β-glucoside system in *Escherichia coli* K-12. J. Bacteriol. **120**:638–650.
284. **Reiner, A. M.** 1977. Xylitol and D-arabitol toxicities due to derepressed fructose, galactitol, and sorbitol phosphotransferases of *Escherichia coli*. J. Bacteriol. **132**:166–173.
285. **Reizer, J., M. J. Novotny, W. Hengstenberg, and M. J. Saier, Jr.** 1984. Properties of ATP-dependent protein kinase from *Streptococcus pyogenes* that phosphorylates a seryl residue in HPr, a phosphocarrier protein of the phosphotransferase system. J. Bacteriol. **160**:333–340.
286. **Reizer, J., M. J. Novotny, C. Panos, and M. H. Saier, Jr.** 1983. Mechanism of inducer expulsion in *Streptococcus pyogenes*: a two-step process activated by ATP. J. Bacteriol. **156**:354–361.
287. **Reizer, J., M. J. Novotny, I. Stuiver, and M. H. Saier, Jr.** 1984. Regulation of glycerol uptake by the phosphoenolpyruvate-sugar phosphotransferase system in *Bacillus subtilis*. J. Bacteriol. **159**:243–250.
288. **Reizer, J., and C. Panos.** 1980. Regulation of β-galactoside phosphate accumulation in *Streptococcus pyogenes* by an expulsion mechanism. Proc. Natl. Acad. Sci. U.S.A. **77**:5497–5501.
289. **Reizer, J., and M. H. Saier, Jr.** 1983. Involvement of lactose enzyme II of the phosphotransferase system in rapid expulsion of free galactosides from *Streptococcus pyogenes*. J. Bacteriol. **156**:236–242.
290. **Rephaeli, A. W., and M. H. Saier, Jr.** 1978. Kinetic analysis of the sugar phosphate:sugar transphosphorylation reaction catalyzed by the glucose Enzyme II complex of the bacterial phosphotransferase system. J. Biol. Chem. **253**:7595–7597.
291. **Rephaeli, A. W., and M. H. Saier, Jr.** 1980. Substrate specificity and kinetic characterization of sugar uptake and phosphorylation, catalyzed by the phosphotransferase system in *Salmonella typhimurium*. J. Biol. Chem. **255**:8585–8591.
292. **Rephaeli, A. W., and M. H. Saier, Jr.** 1980. Regulation of genes coding for enzyme constituents of the bacterial phosphotransferase system. J. Bacteriol. **141**:658–663.
293. **Reynolds, A. E., J. Felton, and A. Wright.** 1981. Insertion of DNA activates the cryptic *bgl* operon in *E. coli* K12. Nature (London) **293**:625–629.
294. **Riley, M., and A. Anilionis.** 1978. Evolution of the bacterial genome. Annu. Rev. Microbiol. **32**:519–560.
295. **Robillard, G. T.** 1982. The enzymology of the bacterial phosphoenolpyruvate-dependent sugar transport system. Mol. Cell. Biochem. **46**:3–24.
296. **Robillard, G. T., G. Dooijewaard, and J. Lolkema.** 1979. *Escherichia coli* phosphoenolpyruvate dependent phosphotransferase system. Complete purification of Enzyme I by hydrophobic interaction chromatography. Biochemistry **18**:2984–2989.
297. **Robillard, G. T., and W. N. Konings.** 1981. Physical mechanism for regulation of phosphoenolpyruvate-dependent glucose transport activity in *Escherichia coli*. Biochemistry **20**:5025–5032.
298. **Robillard, G. T., and W. N. Konings.** 1982. A hypothesis for the role of dithiol-disulfide interchange in solute transport and energy-transducing processes, Eur. J. Biochem. **127**:597–604.
299. **Robillard, G. T., and R. G. Lageveen.** 1982. Non-vectorial phosphorylation by the bacterial PEP-dependent phosphotransferase system is an artifact of spheroplast and membrane vesicle preparation procedures. FEBS Lett. **147**:143–148.
300. **Roehl, R. A., and P. V. Phibbs, Jr.** 1982. Characterization and genetic mapping of fructose phosphotransferase mutations in *Pseudomonas aeruginosa*. J. Bacteriol. **149**:897–905.
301. **Roehl, R. A., and T. Vinopal.** 1980. Genetic locus, distant from *ptsM*, affecting enzyme IIA/IIB function in *Escherichia coli* K-12. J. Bacteriol. **142**:120–130.
302. **Romano, A. H., J. D. Trifone, and M. Brustolon.** 1979. Distribution of the phosphoenolpyruvate:glucose phosphotransferase system in fermentative bacteria. J. Bacteriol. **139**:93–97.
303. **Roossien, F. F., M. Blaauw, and G. T. Robillard.** 1984. Kinetics and subunit interaction of the mannitol-specific Enzyme II of the *Escherichia coli* phosphoenolpyruvate-dependent phosphotransferase system. Biochemistry **23**:4934–4939.
304. **Roossien, F. F., and G. T. Robillard.** 1984. Vicinal dithiol-disulfide distribution in the *Escherichia coli* mannitol specific carrier Enzyme II^{Mtl}. Biochemistry **23**:211–215.
305. **Roossien, F. F., and G. T. Robillard.** 1984. Mannitol-specific carrier protein from *Escherichia coli* phosphoenolpyruvate-dependent phosphotransferase system can be extracted as a dimer from the membrane. Biochemistry **23**:5682–5685.
306. **Rose, S. P., and C. F. Fox.** 1971. The β-glucoside system of *Escherichia coli*. II. Kinetic evidence for a phosphoryl-Enzyme II intermediate. Biochem. Biophys. Res. Commun. **45**:376–380.
307. **Roseman, S.** 1969. The transport of carbohydrates by a bacterial phosphotransferase system. J. Gen. Physiol. **54**:138s–180s.
308. **Rosenberg, H., S. M. Pearce, C. M. Hardy, and P. A. Jacomb.** 1984. Rapid turnover of mannitol-1-phosphate in *Escherichia coli*. J. Bacteriol. **158**:63–68.
309. **Rotman, B., A. K. Ganesan, and R. Guzman.** 1968. Transport systems for galactose and galactosides in *Escherichia coli*. II. Substrate and inducer specificities. J. Mol. Biol. **36**:247–260.
310. **Roy, A., A. Danchin, E. Joseph, and A. Ullmann.** 1983. Two functional domains in adenylate cyclase of *Escherichia coli*. J. Mol. Biol. **165**:197–202.
311. **Rusina, O. Yu., and V. N. Gershanovitch.** 1983. Mapping of mutations within genes coding for Enzyme I and HPr protein of the phosphoenolpyruvate-dependent phosphotransferase system of *Escherichia coli* K12. II. Mapping of *ptsH* mutations within the gene. Genetika **19**:397–405.
312. **Rusina, O. Yu., T. S. Ilyina, and V. N. Gershanovitch.** 1981. Mapping of mutations within genes coding for Enzyme I and HPr of the phosphoenolpyruvate-dependent phosphotransferase system of *Escherichia coli* K12. I. Mapping of *ptsI* intragenic mutations. Genetika **17**:1771–1783.
313. **Saier, M. H., Jr.** 1977. Bacterial phosphoenolpyruvate:sugar phosphotransferase systems: structural, functional, and evolutionary interrelationships. Bacteriol. Rev. **41**:856–871.
314. **Saier, M. H., Jr.** 1980. Catalytic activities associated with the enzymes II of the bacterial phosphotransferase system. J. Supramol. Struct. **14**:281–294.
315. **Saier, M. H., Jr., F. G. Bromberg, and S. Roseman.** 1973. Characterization of constitutive galactose permease mutants in *Salmonella typhimurium*. J. Bacteriol. **113**:512–514.
316. **Saier, M. H., Jr., D. F. Cox, and E. G. Moczydlowski.** 1977. Sugar phosphate:sugar transphosphorylation coupled to exchange group translocation catalyzed by the Enzyme II complexes of the phosphoenolpyruvate:sugar phosphotransferase system in membrane vesicles of *Escherichia coli*. J. Biol. Chem. **252**:8908–8916.
317. **Saier, M. H., Jr., and B. U. Feucht.** 1975. Coordinate regulation of adenylate cyclase and carbohydrate permeases by the phosphoenolpyruvate:sugar phosphotransferase system in *Sal-*

monella typhimurium. J. Biol. Chem. **250**:7078–7080.

318. **Saier, M. H., Jr., B. U. Feucht, and L. J. Hofstadter.** 1976. Regulation of carbohydrate uptake and adenylate cyclase activity mediated by the enzymes II of the phosphoenolpyruvate:sugar phosphotransferase system in *Escherichia coli*. J. Biol. Chem. **251**:883–892.
319. **Saier, M. H., Jr., B. U. Feucht, and M. T. McCaman.** 1975. Regulation of intracellular adenosine cyclic 3′:5′-monophosphate levels in *Escherichia coli* and *Salmonella typhimurium*. Evidence for energy-dependent excretion of the cyclic nucleotide. J. Biol. Chem. **250**:7593–7601.
320. **Saier, M. H., Jr., B. U. Feucht, and W. K. Mora.** 1977. Sugar phosphate:sugar transphosphorylation and exchange group translocation catalyzed by the Enzyme II complexes of the bacterial phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **252**:8899–8907.
321. **Saier, M. H., Jr., B. U. Feucht, and S. Roseman.** 1971. Phosphoenolpyruvate-dependent fructose phosphorylation in photosynthetic bacteria. J. Biol. Chem. **246**:7819–7821.
322. **Saier, M. H., Jr., D. K. Keeler, and B. U. Feucht.** 1982. Physiological desensitization of carbohydrate permeases and adenylate cyclase to regulation by the phosphoenolpyruvate:sugar phosphotransferase system in *Escherichia coli* and *Salmonella typhimurium*. J. Biol. Chem. **257**:2509–2517.
323. **Saier, M. H., Jr., and E. G. Moczydlowski.** 1978. The regulation of carbohydrate transport in *Escherichia coli* and *Salmonella typhimurium*, p. 103–125. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, Inc., New York.
324. **Saier, M. H., Jr., M. J. Newman, and A. W. Rephaeli.** 1977. Properties of a phosphoenol-pyruvate:mannitol phosphotransferase system in *Spirochaeta aurantia*. J. Biol. Chem. **252**:8890–8898.
325. **Saier, M. H., Jr., M. H. Novotny, D. Comeau-Fuhrman, T. Osumi, and J. D. Desai.** 1983. Cooperative binding of the sugar substrates and allosteric regulatory protein (enzyme III^{Glc} of the phosphotransferase system) to the lactose and melibiose permeases in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **155**:1351–1357.
326. **Saier, M. H., Jr., and S. Roseman.** 1972. Inducer exclusion and repression of enzyme synthesis in mutants of *Salmonella typhimurium* defective in Enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **247**:972–975.
327. **Saier, M. H., Jr., and S. Roseman.** 1976. Sugar transport. The *crr* mutation: its effect on repression of enzyme synthesis. J. Biol. Chem. **251**:6598–6605.
328. **Saier, M. H., Jr., and S. Roseman.** 1976. Sugar transport. Inducer exclusion and regulation of the melibiose, maltose, glycerol, and lactose transport systems by the phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **251**:6606–6615.
329. **Saier, M. H., Jr., and M. R. Schmidt.** 1981. Vectorial and nonvectorial transphosphorylation catalzyed by enzymes II of the bacterial phosphotransferase system. J. Bacteriol. **145**:391–397.
330. **Saier, M. H., Jr., M. R. Schmidt, and P. Lin.** 1980. Phosphoryl exchange reaction catalyzed by enzyme I of the bacterial phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **255**:8579–8584.
331. **Saier, M. H., Jr., and R. D. Simoni.** 1976. Regulation of carbohydrate uptake in gram positive bacteria. J. Biol. Chem. **251**:893–894.
332. **Saier, M. H., Jr., R. D. Simoni, and S. Roseman.** 1970. The physiological behaviour of Enzyme I and heat-stable protein mutants of a bacterial phosphotransferase system. J. Biol. Chem. **245**:5870–5873.
333. **Saier, M. H., Jr., R. D. Simoni, and S. Roseman.** 1976. Sugar transport. Properties of mutant bacteria defective in proteins of the phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **251**:6584–6597.
334. **Saier, M. H., Jr., and J. T. Staley.** 1977. Phosphoenolpyruvate:sugar phosphotransferase system in *Ancalomicrobium adetum*. J. Bacteriol. **131**:716–718.
335. **Saier, M. H., Jr., H. Straud, L. S. Massman, J. J. Judice, M. J. Newman, and B. U. Feucht.** 1978. Permease-specific mutations in *Salmonella typhimurium* and *Escherichia coli* that release the glycerol, maltose, melibiose, and lactose transport systems from regulation by the phosphoenolpyruvate:sugar phosphotransferase system. J. Bacteriol. **133**:1358–1367.
336. **Saier, M. H., Jr., W. S. Young, and S. Roseman.** 1971. Utilization and transport of hexoses by mutant strains of *Salmonella typhimurium* lacking Enzyme I of the phosphoenolpyruvate-dependent phosphotransferase system. J. Biol. Chem. **246**:5838–5840.
337. **Sanders, R., and D. McGeoch.** 1973. A mutant transcription factor that is activated by 3′-5′-cyclic guanosine monophosphate. Proc. Natl. Acad. Sci. U.S.A. **70**:1017–1021.
338. **Sanderson, K. E., and J. R. Roth.** 1983. Linkage map of *Salmonella typhimurium*, edition VI. Microbiol. Rev. **47**:410–453.
339. **Sarno, M. V., L. G. Tenn, A. Desai, A. M. Chin, F. C. Grenier, and M. H. Saier, Jr.** 1984. Genetic evidence for glucitol-specific enzyme III, an essential phosphocarrier protein of the *Salmonella typhimurium* glucitol phosphotransferase system. J. Bacteriol. **157**:953–955.
340. **Schäfer, A., O. Schrecker, and W. Hengstenberg.** 1981. The staphylococcal phosphoenolpyruvate-dependent phosphotransferase system. Purification and characterization of the galactoside-specific membrane component Enzyme II. Eur. J. Biochem. **113**:289–294.
341. **Schaefler, S.** 1967. Inducible system for the utilization of β-glucosides in *Escherichia coli*. I. Active transport and utilization of β-glucosides. J. Bacteriol. **93**:254–263.
342. **Schleifer, K.-H., A. Hartinger, and F. Götz.** 1978. Occurrence of D-tagatose-6-phosphate pathway of D-galactose metabolism among *Streptococci*. FEMS Microbiol. Lett. **3**:9–11.
343. **Schmid, K., M. Schupfner, and R. Schmitt.** 1982. Plasmid-mediated uptake and metabolism of sucrose by *Escherichia coli* K-12. J. Bacteriol. **151**:68–76.
344. **Scholte, B. J., and P. W. Postma.** 1980. Mutation in the *crp* gene of *Salmonella typhimurium* which interferes with inducer exclusion. J. Bacteriol. **141**:751–757.
345. **Scholte, B. J., and P. W. Postma.** 1981. Competition between two pathways for sugar uptake by the phosphoenolpyruvate-dependent sugar phosphotransferase system in *Salmonella typhimurium*. Eur. J. Biochem. **114**:51–58.
346. **Scholte, B. J., A. R. Schuitema, and P. W. Postma.** 1981. Isolation of III^{Glc} of the phosphoenolpyruvate-dependent glucose phosphotransferase system of *Salmonella typhimurium*. J. Bacteriol. **148**:257–264.
347. **Scholte, B. J., A. R. Schuitema, and P. W. Postma.** 1982. Characterization of factor III^{Glc} in catabolite repression-resistant (*crr*) mutants of *Salmonella typhimurium*. J. Bacteriol. **149**:576–586.
348. **Shattuck-Eidens, D. M., and R. J. Kadner.** 1983. Molecular cloning of the *uhp* region and evidence for a positive activator for expression of the hexose phosphate transport system of *Escherichia coli*. J. Bacteriol. **155**:1062–1070.
349. **Shibuya, M., Y. Takebe, and Y. Kaziro.** 1977. A possible involvement of *cya* gene in the synthesis of cyclic guanosine 3′:5′-monophosphate in *E. coli*. Cell **12**:521–528.
350. **Shioi, J.-I., R. J. Galloway, M. Niwano, R. E. Chinnock, and B. L. Taylor.** 1982. Requirement of ATP in bacterial chemotaxis. J. Biol. Chem. **257**:7969–7975.
351. **Simoni, R. D., J. B. Hays, T. Nakazawa, and S. Roseman.** 1973. Sugar transport. VI. Phosphoryl transfer in the lactose phosphotransferase system of *Staphylococcus aureus*. J. Biol. Chem. **248**:957–965.
352. **Simoni, R. D., T. Nakazawa, J. B. Hays, and S. Roseman.** 1973. Sugar transport. IV. Isolation and characterization of the lactose phosphotransferase system in *Staphylococcus aureus*. J. Biol. Chem. **248**:932–940.
353. **Simoni, R. D., and S. Roseman.** 1973. Sugar transport. VII. Lactose transport in *Staphylococcus aureus*. J. Biol. Chem. **248**:966–976.
354. **Slater, A. C., M. C. Jones-Mortimer, and H. L. Kornberg.**

1981. L-sorbose phosphorylation in *Escherichia coli* K-12. Biochim. Biophys. Acta **646**:365–367.
355. **Slee, A. M., and J. M. Tanzer.** 1982. Sucrose transport by *Streptococcus mutans*. Evidence for multiple transport systems. Biochim. Biophys. Acta **692**:415–424.
356. **Smith, H. W., and Z. Parsell.** 1975. Transmissible substrate-utilizing ability in enterobacteria. J. Gen. Microbiol. **87**:129–140.
357. **Sobek, H. M., K. Stüber, K. Beyreuther, W. Hengstenberg, and J. Deutscher.** 1984. Staphylococcal phosphoenolpyruvate-dependent phosphotransferase system: purification and characterization of a defective lactose-specific factor III protein. Biochemistry **23**:4460–4464.
358. **Solomon, E., and E. C. C. Lin.** 1972. Mutations affecting the dissimilation of mannitol by *Escherichia coli* K-12. J. Bacteriol. **111**:566–574.
359. **Solomon, E., K. Miyai, and E. C. C. Lin.** 1973. Membrane translocation of mannitol in *Escherichia coli* without phosphorylation. J. Bacteriol. **114**:723–728.
360. **Sprenger, G. A., and J. W. Lengeler.** 1984. L-Sorbose metabolism in *Klebsiella pneumoniae* and Sor$^+$ derivatives of *Escherichia coli* K-12 and chemotaxis toward sorbose. J. Bacteriol. **157**:39–45.
361. **St. Martin, E. J., and C. L. Wittenberger.** 1979. Characterization of a phosphoenolpyruvate-dependent sucrose phosphotransferase system in *Streptococcus mutans*. Infect. Immun. **24**:865–868.
362. **Stock, J., A. M. Maderis, and D. E. Koshland, Jr.** 1981. Bacterial chemotaxis in the absence of receptor carboxylmethylation. Cell **27**:37–44.
363. **Stock, J. B., E. B. Waygood, N. D. Meadow, P. W. Postma, and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. The glucose receptors of the *Salmonella typhimurium* phosphotransferase system. J. Biol. Chem. **257**:14543–14552.
364. **Struhl, K., and R. W. Davis.** 1980. Conservation and DNA sequence arrangement of the DNA polymerase I gene region from *Klebsiella aerogenes*, *Klebsiella pneumoniae* and *Escherichia coli*. J. Mol. Biol. **141**:343–368.
365. **Tanaka, S., D. G. Fraenkel, and E. C. C. Lin.** 1967. The enzymatic lesion of strain MM6, a pleiotropic carbohydrate-negative mutant of *Escherichia coli*. Biochem. Biophys. Res. Commun. **27**:63–67.
366. **Tanaka, S., S. A. Lerner, and E. C. C. Lin.** 1967. Replacement of a phosphoenolpyruvate-dependent phosphotransferse by a nicotinamide adenine dinucleotide-linked dehydrogenase for the utilization of mannitol. J. Bacteriol. **93**:642–648.
367. **Tanaka, S., and E. C. C. Lin.** 1967. Two classes of pleiotropic mutants of *A. aerogenes* lacking components of PEP phosphotransferase system. Proc. Natl. Acad. Sci. U.S.A. **57**:913–919.
368. **Tao, M., and F. Lipmann.** 1969. Isolation of adenyl cyclase from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **63**:86–92.
369. **Thompson, J., and B. M. Chassy.** 1981. Uptake and metabolism of sucrose by *Streptococcus lactis*. J. Bacteriol. **147**:543–551.
370. **Thompson, J., and B. M. Chassy.** 1982. Novel phosphoenolpyruvate-dependent futile cycle in *Streptococcus lactis*: 2-deoxy-D-glucose uncouples energy production from growth. J. Bacteriol. **151**:1454–1465.
371. **Thompson, J., and B. M. Chassy.** 1983. Intracellular hexose-6-phosphate:phosphohydrolase from *Streptococcus lactis*: purification, properties, and function. J. Bacteriol. **156**:70–80.
372. **Thompson, J., and B. M. Chassy.** 1985. Intracellular phosphorylation of glucose analogs via the phosphoenolpyruvate: mannose-phosphotransferase system in *Streptococcus lactis*. J. Bacteriol. **162**:224–234.
373. **Thompson, J., and M. H. Saier, Jr.** 1981. Regulation of methyl-β-D-thiogalactopyranoside-6-phosphate accumulation in *Streptococcus lactis* by exclusion and expulsion mechanisms. J. Bacteriol. **146**:885–894.
374. **Tyler, B., and B. Magasanik.** 1970. Physiological basis of transient repression of catabolic enzymes in *Escherichia coli*. J. Bacteriol. **102**:411–422.
375. **Ullmann, A., and A. Danchin.** 1983. Role of cyclic AMP in bacteria. Adv. Cyclic Nucleotide Res. **15**:2–53.
376. **Voloshin, A. G., N. A. Belkina, and G. I. Bourd.** 1983. Mechanism of catabolite repression in *Escherichia coli*: interaction between transport proteins and adenylate cyclase. Biokhimiya **48**:1624–1633.
377. **Wagner, E. F., J. D. Fabricant, and M. Schweiger.** 1979. A novel ATP-driven glucose transport system in *Escherichia coli*. Eur. J. Biochem. **102**:231–236.
378. **Walsh, C. T., and H. R. Kaback.** 1973. Vinylglycolic acid. An inactivator of the phosphoenolpyruvate-phosphate transferase system in *Escherichia coli*. J. Biol. Chem. **248**:5456–5462.
379. **Walter, R. W., Jr., and R. L. Anderson.** 1973. Evidence that the inducible phosphoenolpyruvate:D-fructose 1-phosphate phosphotransferase system of *Aerobacter aerogenes* does not require "HPr". Biochem. Biophys. Res. Commun. **52**:93–97.
380. **Wang, J. Y. J., D. O. Clegg, and D. E. Koshland, Jr.** 1981. Molecular cloning and amplification of the adenylate cyclase gene. Proc. Natl. Acad. Sci. U.S.A. **78**:4684–4688.
381. **Wang, R. J., H. G. Morse, and M. L. Morse.** 1969. Carbohydrate accumulation and metabolism in *Escherichia coli*: the close linkage and chromosomal location of *ctr* mutations. J. Bacteriol. **98**:605–610.
382. **Wang, R. J., H. G. Morse, and M. L. Morse.** 1970. Carbohydrate accumulation and metabolism in *Escherichia coli*: characteristics of the reversions of *ctr* mutations. J. Bacteriol. **104**:1318–1324.
383. **Wang, R. J., and M. L. Morse.** 1968. Carbohydrate accumulation and metabolism in *Escherichia coli*. Description of pleiotropic mutants. J. Mol. Biol. **32**:59–66.
384. **Wanner, B. L., R. Kodaira, and F. C. Neidhardt.** 1978. Regulation of *lac* operon expression: reappraisal of the theory of catabolite repression. J. Bacteriol. **136**:947–954.
385. **Waygood, E. B.** 1980. Resolution of the phosphoenolpyruvate:fructose phosphotransferase system of *Escherichia coli* into two components; Enzyme II fructose and fructose-induced HPr-like protein (FPr). Can. J. Biochem. **58**:1144–1146.
386. **Waygood, E. B., R. L. Mattoo, and K. G. Peri.** 1984. Phosphoproteins and the phosphoenolpyruvate:sugar phosphotransferase system in *Salmonella typhimurium* and *Escherichia coli*: evidence for III^{Man}, III^{Fru}, $III^{Glucitol}$, and the phosphorylation of Enzyme $II^{Mannitol}$ and Enzyme $II^{N\text{-acetylglucosamine}}$. J. Cell. Biochem. **25**:139–159.
387. **Waygood, E. B., N. D. Meadow, and S. Roseman.** 1979. Modified assay procedures for the phosphotransferase system in enteric bacteria. Anal. Biochem. **95**:293–304.
388. **Waygood, E. B., and T. Stevens.** 1980. Enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) of *Escherichia coli*—purification to homogeneity and some properties. Can. J. Biochem. **58**:40–48.
389. **Weigel, N., M. A. Kukuruzinska, A. Nakazawa, E. B. Waygood, and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Phosphoryl transfer reactions catalyzed by Enzyme I of *Salmonella typhimurium*. J. Biol. Chem. **257**:14477–14491.
390. **Weigel, N., D. A. Powers, and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Primary structure and active site of a general phosphocarrier protein (HPr) from *Salmonella typhimurium*. J. Biol. Chem. **257**:14499–14509.
391. **Weigel, N., E. B. Waygood, M. A. Kukuruzinska, A. Nakazawa, and S. Roseman.** 1982. Sugar transport by the bacterial phosphotransferase system. Isolation and characterization of Enzyme I from *Salmonella typhimurium*. J. Biol. Chem. **257**:14461–14469.
392. **White, R. J.** 1970. The role of the phosphoenolpyruvate phosphotransferase system in the transport of N-acetyl-D-glucosamine by *Escherichia coli*. Biochem. J. **118**:89–92.
393. **Willis, D. K., B. E. Uhlin, K. S. Amini, and A. J. Clark.** 1981. Physical mapping of the *srl recA* region of *Escherichia coli*: analysis of Tn10 generated insertions and deletions. Mol. Gen. Genet. **183**:497–504.
394. **Wilson, D. B.** 1978. Cellular transport mechanisms. Annu.

Rev. Biochem. **47**:933–965.
395. **Winkler, H. H.** 1970. Compartmentation in the induction of the hexose-6-phosphate transport system of *Escherichia coli*. J. Bacteriol. **101**:470–475.
396. **Winkler, H. H.** 1971. Efflux and the steady state in α-methylglucoside transport in *Escherichia coli*. J. Bacteriol. **106**:362–368.
397. **Winkler, H. H., and T. H. Wilson.** 1967. Inhibition of β-galactoside transport by substrates of the glucose transport system in *Escherichia coli*. Biochim. Biophys. Acta **135**:1030–1051.
398. **Woodward, M. J., and H. P. Charles.** 1982. Genes for L-sorbose utilization in *Escherichia coli*. J. Gen. Microbiol. **128**:1969–1980.
399. **Woodward, M. J., and H. P. Charles.** 1983. Polymorphism in *Escherichia coli*: *rtl*, *atl* and *gat* regions behave as chromosomal alternatives. J. Gen. Microbiol. **129**:75–84.
400. **Yang, J. K., R. W. Bloom, and W. Epstein.** 1979. Catabolite and transient repression in *Escherichia coli* do not require enzyme I of the phosphotransferase system. J. Bacteriol. **138**:275–279.
401. **Yang, J. K., and W. Epstein.** 1983. Purification and characterization of adenylate cyclase from *Escherichia coli* K-12. J. Biol. Chem. **259**:3750–3758.