

Two Systems for the Uptake of Phosphate in *Escherichia coli*

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Mutants of *Escherichia coli* K-12 were constructed such that each possessed one single major system for phosphate transport. A comparison of these strains showed that one of the systems (PIT) was fully constitutive, required no binding protein, and operated in spheroplasts. It permitted the complete exchange of intracellular phosphate with extracellular phosphate (or arsenate) and was completely inhibited by uncouplers. The other system, PST, was repressible by phosphate concentrations above 1 mM, required the phosphate-binding protein for full activity, and did not operate in spheroplasts. It catalyzed very little exchange between internal and external phosphate and was resistant to uncouplers. The maximal velocities attained by the two systems were approximately the same, but the affinity for phosphate in the PST system was greater by two orders of magnitude. In strains in which both systems were fully operative, the initial rates of uptake were nearly additive, and the systems appeared to interact with a common intracellular phosphate pool.

Phosphate transport in *Escherichia coli* has been studied by a number of workers in recent years (6, 10, 12, 13, 16-18, 22). In several aspects of the subject, these studies have produced conflicting findings that were difficult to reconcile. A possible explanation was provided by Willsky et al. (22), who demonstrated the presence of two major systems for phosphate uptake in *E. coli* K-12. The first system, designated the "PST system," depends upon the presence of the wild-type gene *phoT* (ca. min 82 on the *E. coli* linkage map [1]) for full activity (22). Another gene (*phoS*), closely linked to *phoT*, codes for the phosphate-binding protein, which according to recent evidence (7), forms an essential part of the complete transport process in the PST system. Each of these loci is involved in the regulation of alkaline phosphatase synthesis in *E. coli* (5). The involvement of a third gene, *pst*, in the PST system has also been claimed (1, 20, 22), but its function is unknown. The second system for phosphate transport in *E. coli* has been designated the "PIT system" (23), for which one gene, *pit*, mapping at 62 min, has been described (20).

The demonstration of two discrete transport systems made it clear that most of the previous work with strains of *E. coli* K-12 dealt with a composite of the two systems, the contribution of each being potentially variable depending on the strain and growth conditions. In particular, we felt that the presence, in one cell, of two systems for phosphate uptake, possibly different in several aspects, may have contributed to

the fact that earlier work from this laboratory, dealing with phosphate uptake in cold-shocked cells (12), could not be reproduced consistently. Indeed, the effect of cold shock on phosphate transport in strain K-12 was found to be variable (17). In addition, recent studies on the nature of energy coupling to phosphate transport in *E. coli* have produced conflicting findings. Thus, while some evidence (18) pointed to coupling to the energized membrane state (11), other results (16) suggested that phosphate bond energy was involved directly, in a manner similar to that of other shock-sensitive systems (3) and, particularly, to that described by Harold and Spitz (9) for *Streptococcus faecalis*.

In view of this, we examined several aspects of phosphate transport in *E. coli* under conditions in which the two systems could be studied separately. This paper describes the construction of strains, each possessing one single, major system for phosphate transport, and provides a comparison of their properties.

MATERIALS AND METHODS

The chemicals used were of the highest purity available commercially. Triethanolamine (Fluka Purissimum grade) was further purified by crystallization of the hydrochloride from ethanol. Lysozyme was a product of Fluka, and carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) was from Calbiochem, La Jolla, Calif. Carrier-free [³²P]orthophosphate (³²P_i) was from the Australian Atomic Energy Commission, Lucas Heights, New South Wales.

Media. Cells were grown overnight with shaking at 37°C in a medium (pH 7.0) containing: K₂HPO₄,

30 mM; NaH₂PO₄, 20 mM; MgSO₄, 0.4 mM; and (NH₄)₂SO₄, 8 mM. Sterile solutions of growth supplements were added, where appropriate, at the following final concentrations: D-glucose, 20 mM; potassium DL-lactate, 25 mM; thiamine, 3 μM; arginine, 1 mM; and 2,3-dihydroxybenzoate, 10 μM. Growth experiments in the presence of arsenate were carried out in the TS medium of Echols et al. (5), with glucose and essential supplements. Phosphate-free buffered "uptake" medium (pH 6.9) contained: triethanolamine-HCl, 50 mM; KCl, 15 mM; (NH₄)₂SO₄, 10 mM; and MgSO₄, 1 mM. The "wash" solution used in uptake assays was similar to the uptake medium, except that the triethanolamine and KCl concentrations were 10 and 100 mM, respectively.

Genetic techniques. The strains used, which were all derivatives of *E. coli* K-12, are listed in Table 1. Transductions were carried out with phage Pl_{kc} by the method of Pittard (15). Transduction of *phoS* or *phoT* markers was carried out into *ilv* recipients. Selection was made for *ilv*⁺, followed by screening for strains constitutive for alkaline phosphatase (22).

Preparation of cells for uptake. Cells were harvested in the stationary phase after overnight growth (16 h), except where stated otherwise. Cells of strain AN710 and its derivatives were washed three times with the uptake medium and suspended at an optical density (660 nm) of 0.35. Cells of strain K10 and its derivatives were washed twice as described above and resuspended in the uptake medium at an optical density (660 nm) of 0.35, and the same carbon sources as used for growth were added. The cells were shaken for 2 h to induce maximal phosphate uptake rates (see below), washed three times in uptake medium to remove any residual carbon source, and suspended at an optical density (660 nm) of 0.35. These suspensions were stored in a refrigerator, for up to 2 h, until required. Carbon sources and other additions were made where appropriate, and the suspensions were shaken at 37°C for 5 min before commencing uptake assays.

Measurement of phosphate uptake. Phosphate

uptake was measured as described elsewhere (18), except that the wash solution used was as described above.

Analysis of intracellular acid-soluble phosphate. Samples of cells incubated with ³²P in the standard transport assay were filtered through cellulose nitrate membranes (pore size, 0.45 μm) and washed as usual. Each membrane was immediately placed into 2.5 ml of 0.75 N HClO₄-0.1 mM H₃PO₄ at 0°C. Extraction of acid-soluble material was complete after 10 min of gentle agitation. The acid extract was centrifuged to remove bacteria, which became dislodged from the filter. Samples (1.0 ml) of the clear extract were mixed with 50 μl of 5% ammonium molybdate and shaken with 2.0 ml of isobutanol-benzene (1:1, vol/vol). Each layer was counter-washed twice, made to 10.0 ml with ethanol, and counted by using Cerenkov radiation. All samples were compensated with quantities of "aqueous" and "butanol-benzene" phases to make them chemically similar for counting, and appropriate blanks and standards were also counted.

Phosphate uptake in spheroplasts. The preparation of spheroplasts and the method used for assaying phosphate uptake in suspensions of spheroplasts were as described elsewhere (7).

Analysis of kinetic data. Computer-fitting of data from kinetic experiments was done by the method of least squares according to the procedure of Wilkinson (21).

RESULTS

Preparation of bacterial strains. Willsky et al. (22) reported that strain K10 lacks the PIT system; the PST system can therefore be studied alone in this strain and in mutants derived from it. Other strains of *E. coli* K-12 normally contain both systems, and the introduction of a *phoT* mutation would abolish the activity of the PST system (22), leaving only the PIT system operative in *phoT* mutants. This served as a basis for the assembly and preparation of the strains used in this work (Table 1).

TABLE 1. *E. coli* strains used in this work

Strain designation	P _i transport system(s) present	Relevant genetic markers ^a	Source/comment
K10	PST	<i>pit</i>	B. Bachmann, CGSC 5023 (derivative of Hfr Cavalli [5])
C-31	PST ^b	<i>pit phoS31</i>	A. Garen
C-72	PST ^b	<i>pit phoS72</i>	A. Garen
C-101a	PST	<i>pit phoT101</i>	A. Garen
AN248	PST, PIT	<i>ilv</i>	G. Cox
AN259	PST, PIT	<i>ilv</i> ⁺	G. Cox (transductant from AN248)
AN518	PST	<i>pit ilv</i>	Ultraviolet mutagenesis of strain K10
AN521	PST ^b	<i>pit phoS31</i>	Transduction of relevant alleles into strain AN518
AN524	PST ^b	<i>pit phoS72</i>	Transduction of relevant alleles into strain AN518
AN710	PIT	<i>phoT101</i>	Transduction of relevant alleles into strain AN248
AN796	PIT, PST ^b	<i>phoS72</i>	Transduction of relevant alleles into strain AN248

^a Allele numbers were assigned according to the strain numbers carried by the original strains from Echols et al. (5).

^b The operation of the PST system in these strains is greatly attenuated due to the *phoS* mutation (see text).

Effect of phosphate deprivation on transport rates. Aerobic incubation in the buffered phosphate-free uptake medium produced no change in the transport rate of phosphate in the *phoT101* strain (AN710), but in strain K10, where only the PST system is operative, the rate increased severalfold during the 3-h deprivation period (Fig. 1). It is clear from the effect of chloramphenicol (Fig. 1) that the increase in uptake rate in the PST system during phosphate starvation involves the synthesis of protein and that at least one such protein is the phosphate-binding protein (*phoS* gene product), since phosphate starvation failed to alter the low uptake rate in the *phoS72* mutant.

Transport kinetics. Some kinetic parameters of the two systems differ (Table 2). Whereas maximal velocity values for the PIT system and the fully derepressed PST system are similar, the affinity for the substrate is higher by two orders of magnitude in the PST system than in the PIT system. It is of interest that the loss of the binding protein (*phoS* mutation in the PST system) had little effect on the affinity but reduced the maximal velocity by a factor of about 15. Comparison of the data for strains AN710 and AN796 shows that the binding protein did not play a role in the PIT system.

Inhibition by arsenate. The inhibition of phosphate transport in bacteria by arsenate has been well documented (8, 14, 19), and arsenate

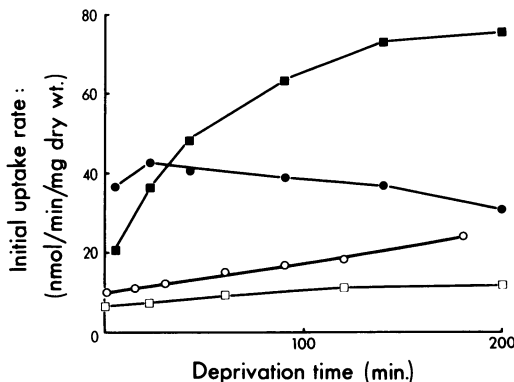


FIG. 1. Derepression of the PST system for phosphate transport in *E. coli*. Cells were grown with glucose as the carbon source and were washed twice in uptake medium containing glucose and other supplements as required (see text). Suspensions of washed cells at an optical density (at 660 nm) of 0.35 were shaken in the same medium at 37°C. Samples were removed at the indicated times for measurement of phosphate uptake rates as described in the text. symbols: ●, strain AN710 (*phoT101*); ■, strain K10 (*pit*); □, strain AN524 (*phoS72 pit*); ○, strain K10 plus chloramphenicol at 50 $\mu\text{g/ml}$.

resistance has been used to select mutants of *E. coli* defective in phosphate transport (2, 12). We found that the growth of strains AN710 (PIT system) and K10 (PST system) was inhibited by arsenate (Table 3). However, the arsenate concentration at which this occurred differed for each strain. Indeed, an arsenate concentration could be chosen (10 mM) that completely inhibited the growth of strain AN710 but only halved the growth rate of strain K10. The kinetic parameters for this inhibition (Table 2) also show a difference: the K_i for arsenate in strain K10 is about 40 times the K_m for phosphate; in strain AN710 it is of the same order of magnitude as the K_m for phosphate. These figures show that, at an arsenate concentration of 10 times the phosphate level in the medium, the toxic anion readily enters the cells via the PIT system but would not be expected to be readily transported by the PST system. The growth rate data in Table 3 are in accordance with this view.

Exchange of intracellular phosphate pool

TABLE 2. Kinetic parameters for phosphate transport in strains of *E. coli* K-12^a

Strain	Relevant genotype	Apparent K_m (μM)	K_i (arsenate) (μM)	V_{max} (nmol of P/min per mg (dry wt))
K10	<i>pit</i>	0.16 \pm 0.02	6.2 \pm 0.9	69 \pm 4
AN521	<i>phoS31 pit</i>	0.18 \pm 0.06		5.4 \pm 0.4
AN524	<i>phoS72 pit</i>	0.34 \pm 0.12		4.1 \pm 0.4
AN710	<i>phoT101</i>	25 \pm 1	31.6 \pm 1.1	60 \pm 2
AN796	<i>phoS72</i>	24 \pm 1.3		60 \pm 2

^a Bacteria were grown in glucose as the carbon source and were prepared as described in the text. The standard uptake assay was modified in that phosphate concentrations ranged from 0.3 to 4.0 μM for strains K10, AN521, and AN524, and between 5.0 and 62.5 μM for AN710 and AN796; initial rates were determined by sampling at 10 and 20 s. The data are given with standard errors of the mean. For the calculation of K_i (arsenate), the experiments were repeated in the presence of arsenate at the concentration ranges 0.5 to 10 and 1 to 50 μM for strains K10 and AN710, respectively. Between 6 and 10 phosphate concentrations were used within each range.

TABLE 3. Effect of arsenate on the growth of *E. coli* K-12 strains affected in phosphate transport

Strain and relevant genetic markers	Mean generation time ^a (h)			
	Control	1 mM arsenate	10 mM arsenate	100 mM arsenate
K10 <i>pit</i>	1.6	1.8	3.9	>10
AN524 <i>pit phoS72</i>	2.7	2.8	5.2	>20
AN710 <i>phoT101</i>	2.2	3.5	>20	>20
AN796 <i>phoS72</i>	1.4	2.0	>20	>20

^a Cells were grown at 37°C to early log phase in the TS medium of Echols et al. (5), supplemented with 1 mM phosphate, 20 mM glucose, and essential nutrients. Arsenate was added at the concentrations shown, and the subsequent growth was monitored.

with extracellular phosphate or arsenate. The exchange of the phosphate pool has been reported from this laboratory (13) for *E. coli* K-12, but the results could not be reproduced consistently with similar strains by others (G. R. Willisky, personal communication) or by ourselves. The reason for this became apparent when the ability to exchange phosphate was compared in strains K10 and AN710 grown with lactate as a carbon source (Fig. 2). The addition of unlabeled P_i or arsenate in a large excess (400 times the extracellular concentration of the added ^{32}P) to a suspension of AN710 cells that had accumulated phosphate for 60 s caused an abrupt loss of the bulk of radioactivity present in the cells, in the subsequent 60 s. Under the same conditions, only about 15% of the radioactivity was lost from strain K10. Examination, in a parallel run, of the intracellular acid-soluble fraction of the cells (see Materials and Methods) revealed that, at the 60-s point, P_i accounted for the major portion of the radioactivity in that fraction: 72% in strain AN710 and 63% in strain K10. Yet, all of the $^{32}P_i$ exchanged in the former, but only 25% did so in the latter. Arsenate and phosphate showed similar patterns in each strain.

It should be noted that this exchange was not observed in strain AN710 grown on glucose. The reason for this is not yet understood and is being investigated.

Effect of the uncoupler CCCP. CCCP affects the two systems in distinctly different fashions (Table 4). The increment in uptake

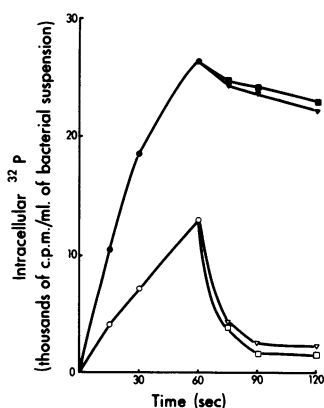


FIG. 2. Exchange of cytoplasmic phosphate with external phosphate or arsenate. Cells grown with lactate as the carbon source and prepared as described in the text were given $^{32}P_i$, and the uptake of phosphate was monitored for 60 s (\square , \bullet). At this time, a 400-fold excess of unlabeled phosphate (\square , \blacksquare) or arsenate (\triangle , \blacktriangle) was added, and the content of ^{32}P in the cells was monitored for a further 60 s. Solid symbols, strain K10; open symbols, strain AN710.

rates due to added glucose was completely abolished by CCCP in strain AN710 (PIT system) but not in K10 (PST system), suggesting coupling of phosphate transport to the "high-energy state" of the membrane in the PIT system but not in the PST system.

Phosphate uptake in spheroplasts. Spheroplast formation, which results in loss of the phosphate-binding protein, should severely affect the PST system but not the PIT system. A comparison of uptake rates (Table 5) in whole cells and spheroplasts of the two strains confirms that uptake rates in spheroplasts of strain K10 were close to zero, whereas spheroplasts of strain AN710 took up phosphate at rates observed for whole cells under the same conditions. Furthermore, the uptake by AN710 spheroplasts was totally abolished by CCCP and, of the ^{32}P taken up in 3 min, 70% was exchangeable with external P_i (data not shown). The total release of alkaline phosphatase

TABLE 4. Effect of CCCP on the rate of phosphate uptake in the two systems

Strain ^a	CCCP ^b	Uptake rate (nmol/min per mg (dry wt))		
		No carbon source	20 mM glucose	Glucose stimulated
AN710 (PIT system)	-	1.8	26.0	24.2
	+	2.3	3.4	1.1
K10 (PST system)	-	15.2	59.4	44.2
	+	7.1	45.9	38.8

^a Cells were grown with 20 mM glucose as a carbon source and were prepared as described in the text.

^b Where indicated, CCCP was added to 40 μ M, 5 min before the addition of ^{32}P , and the cells were shaken at 37°C.

TABLE 5. Rate of P_i uptake in whole cells and spheroplasts of *E. coli*^a

Strain	Uptake rate (nmol/min per mg (dry wt))		Alkaline phosphatase released in spheroplast prep'n (%)	% of uptake rate retained in spheroplasts
	Whole cells	Spheroplasts ^b		
K10 (PST system)	33.2	2.2	92.5	6.6
AN710 (PIT system)	10.1	11.3	100	100

^a The cells were grown to the late log phase, as described in the text, with glucose as the carbon source. Spheroplasts were prepared as described elsewhere (7), and the uptake rates of whole cells and spheroplasts were measured in the presence of 15% sucrose (7), where the rates are considerably lower than in normal uptake medium.

^b In the case of spheroplasts, "milligram (dry weight)" refers to the original cells from which the spheroplasts were prepared.

tase (Table 5) is taken as an indication that both strains had lost the periplasmic proteins during spheroplast formation.

Concomitant operation of the two systems. We used cells of strain AN259, wild-type for the PIT and PST system, to establish whether the rates of phosphate uptake by the two systems were additive and whether the PIT system continued operation when the PST system became derepressed. When these cells were grown to log phase in the usual growth medium (containing 50 mM P_i), the PST system was largely repressed, but under standard assay conditions the cells took up phosphate at a rate of about 45 nmol/min per mg (dry weight) (Table 6). This reflects the activity of the PIT system, since similarly grown cells of strain AN710 (PIT system) showed comparable uptake rates, but cells of strain K10 showed, under the same conditions, a rate of about 13 nmol/min per mg (dry weight), due to repression of the PST system. After 2 h of incubation under conditions of phosphate deprivation, the uptake rates by strains K10 and AN259 rose by some 50 and 42 nmol/min per mg (dry weight), respectively, while that of AN710 remained constant. It would thus appear that, in strain AN259, the PST system was derepressed to an extent comparable to that in strain K10, without greatly affecting the PIT system.

That the PIT system still operated in strain AN259 after phosphate deprivation is also demonstrated by the fact that the fraction of intracellular phosphate that was subject to exchange remained constant and equal to the fraction in strain AN710. This also shows that the phosphate accumulated by both systems enters a common pool, and all of it is accessible to exchange via the PIT system.

DISCUSSION

The present results explain a number of discrepancies in published properties of phosphate

transport in *E. coli*. These differences reflect, in part, the considerable differences in behavior of the two distinct transport systems, PIT and PST.

Our findings confirm the constitutive nature of the PIT transport system. However, in the light of the present findings, the reference to the PST system as a constitutive one (23) needs qualification. Although this system does operate in cells grown on media containing P_i at concentrations of 1 mM or higher, growth on limiting P_i or deprivation of phosphate leads to a severalfold increase in the rate of transport. It is remarkable that under these limiting conditions the concentration of the phosphate-binding protein increases about 100-fold (24). The present work (Fig. 1) shows that the presence of the phosphate-binding protein is essential for the maximal uptake rate in the PST system. The substantial repression of the PST system by growth in 1 mM P_i explains the low V_{max} value for phosphate transport reported earlier by Willsky and Malamy (23). These were close to the rates observed by us for nonstarved cells, whereas in fully derepressed cells the rate was five times that (Table 6).

Another point of discrepancy relates to the results of Kida (10), who found somewhat reduced phosphate uptake rates in *phoT* mutants of strain K10 and negligible uptakes in *phoS* mutants. These results are the opposite of those observed by us and reported by others (22). There are two possible reasons for this discrepancy. First, the mutants used by Kida (10) carry *phoS* and *phoT* alleles different from those used in the present work. Second, as mentioned by Willsky and Malamy (24), the phenotypic expression of the *phoS* mutation may be influenced by the genetic background of the recipient.

The direct involvement of the *phoS* product, the phosphate-binding protein, in the transport of phosphate by the PST system has been ade-

TABLE 6. Effect of phosphate deprivation on the two systems operating singly or together

Strain ^a	Transport system present	Phosphate uptake rate ^b		Exchangeable-P fraction ^c	
		Before P deprivation	After P deprivation ^d	Before P deprivation	After P deprivation
K10	PST	13.5	63.4	0.18	0.19
AN710	PIT	38.7	38.5	0.73	0.72
AN259	PST, PIT	45.6	87.2	0.68	0.65

^a Cells were grown to mid-log phase, with lactate as the carbon source, and were prepared as described in the text.

^b Initial rates: Nanomoles of phosphate per minute per milligram (dry weight).

^c Fraction of ³²P accumulated in 30 s that was lost in the subsequent 60 s after the addition of a 400-fold excess of unlabeled P_i .

^d P deprivation time was 2 h.

quately demonstrated by the reconstitution of uptake in spheroplasts (7). The nature of the mechanism is, as yet, unknown. The mechanism of the control of alkaline phosphatase synthesis by the *phoS* (or *phoT*) products is also unclear. It is unlikely that it is exerted through their influence on intracellular phosphate levels, since it is clear that *E. coli* K-12 strains that carry either a *phoS* or *phoT* mutation have fully derepressed alkaline phosphatase levels while maintaining normal rates of phosphate transport through the PIT system. The two systems appear to operate with a common intracellular phosphate pool (Table 6).

The transport kinetics shown in Table 2 raise several points of interest. In contrast to Willsky and Malamy (23), we found that the introduction of the *phoS* mutation (any one of several different alleles) increased neither the K_m value for phosphate nor the cells' sensitivity to arsenate. A possible explanation for this difference between K_m values may be that we used cells fully induced by phosphate deprivation for 2 h, whereas they used cells grown in 1 mM P_i , in which the *phoS* product was present only at about 1% of maximal values (24).

The present results clearly demonstrate that the strains containing the PIT system are consistently more sensitive to arsenate than the K10 derivatives (Table 3). The different kinetic constants for phosphate and arsenate in these systems are also consistent with the observed inhibition by arsenate of the two strains. In this respect, our results fully agree with those of Willsky and Malamy (24).

An interesting feature of the PIT system is that it catalyzes a rapid exchange of intracellular P_i for extracellular P_i or arsenate. The reason for the repression of this phenomenon in glucose-grown cells is unknown.

The preliminary results on energy coupling suggest that the two transport systems are energized differently, and this is in accord with the observation that the PST is a "shockable" system (3), involving a binding protein (7), whereas the PIT is not. A more detailed investigation of the bioenergetics of phosphate uptake by the two systems, involving mutants defective in energy coupling, is in progress (H. Rosenberg, R. G. Gerdes, and F. M. Harold, manuscript in preparation).

The results shown in Table 6 leave no doubt that the two systems can operate in concert in suitably induced cells and that each has access to one common pool of intracellular phosphate.

It is of interest that a similar dual system of phosphate transport in yeast was recently described (4).

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LITERATURE CITED

- Bachmann, B. J., K. B. Low, and A. L. Taylor. 1976. Recalibrated linkage map of *Escherichia coli* K-12. *Bacteriol. Rev.* 40:116-167.
- Bennett, R. L., and M. H. Malamy. 1970. Arsenate resistant mutants of *Escherichia coli* and phosphate transport. *Biochem. Biophys. Res. Commun.* 40:496-503.
- Berger, E. A., and L. A. Heppel. 1974. Different mechanisms of energy coupling for the shock-sensitive and shock-resistant amino acid permeases of *Escherichia coli*. *J. Biol. Chem.* 249:7747-7755.
- Blasco, F., G. Ducet, and E. Azoulay. 1976. Mise en évidence de deux systèmes de transport du phosphate chez *Candida tropicalis*. *Biochimie* 58:351-357.
- Echols, H., A. Garen, S. Garen, and A. Torriani. 1961. Genetic control of repression of alkaline phosphatase in *E. coli*. *J. Mol. Biol.* 3:425-438.
- Gerdes, R. G., and H. Rosenberg. 1974. The relationship between the phosphate-binding protein and a regulator gene product from *Escherichia coli*. *Biochim. Biophys. Acta* 351:77-86.
- Gerdes, R. G., K. P. Strickland, and H. Rosenberg. 1977. Restoration of phosphate transport by the phosphate-binding protein in spheroplasts of *Escherichia coli*. *J. Bacteriol.* 131:512-518.
- Harold, F. M., and J. R. Baarda. 1966. Interaction of arsenate with phosphate transport systems in wild-type and mutant *Streptococcus faecalis*. *J. Bacteriol.* 91:2257-2262.
- Harold, F. M., and E. Spitz. 1975. Accumulation of arsenate, phosphate, and aspartate by *Streptococcus faecalis*. *J. Bacteriol.* 122:266-277.
- Kida, S. 1974. The biological function of the R2a regulatory gene for alkaline phosphatase in *Escherichia coli*. *Arch. Biochem. Biophys.* 163:231-237.
- Klein, W. L., and P. D. Boyer. 1972. Energization of active transport by *Escherichia coli*. *J. Biol. Chem.* 247:7257-7265.
- Medveczky, N., and H. Rosenberg. 1970. The phosphate-binding protein of *Escherichia coli*. *Biochim. Biophys. Acta* 211:158-168.
- Medveczky, N., and H. Rosenberg. 1971. Phosphate transport in *Escherichia coli*. *Biochim. Biophys. Acta* 241:494-506.
- Mitchell, P. 1954. Transport of phosphate across the osmotic barrier of *Micrococcus pyogenes*: specificity and kinetics. *J. Gen. Microbiol.* 11:73-82.
- Pittard, J. 1965. Effect of integrated sex factor on transduction of chromosomal genes in *Escherichia coli*. *J. Bacteriol.* 89:680-686.
- Rae, A. S., and K. P. Strickland. 1976. Studies on phosphate transport in *Escherichia coli*. II. Effects of metabolic inhibitors and divalent cations. *Biochim. Biophys. Acta* 433:564-582.
- Rae, A. S., K. P. Strickland, N. Medveczky, and H. Rosenberg. 1976. Studies on phosphate transport in *Escherichia coli*. I. Re-examination of the effect of osmotic and cold shock on phosphate uptake and some attempts to restore uptake with phosphate binding protein. *Biochim. Biophys. Acta* 433:555-563.
- Rosenberg, H., G. B. Cox, J. D. Butlin, and S. J. Gutowski. 1975. Metabolite transport in mutants of *Escherichia coli* K12 defective in electron transport and coupled phosphorylation. *Biochem. J.* 146:417-423.
- Rosenberg, H., and J. M. La Nauze. 1968. The isolation

- of a mutant of *Bacillus cereus* deficient in phosphate uptake. *Biochim. Biophys. Acta* 156:381-388.
20. Sprague, G. F., R. M. Bell, and J. E. Cronan, Jr. 1975. A mutant of *Escherichia coli* auxotrophic for organic phosphates: evidence for two defects in inorganic phosphate transport. *Mol. Gen. Genet.* 143:71-77.
21. Wilkinson, G. N. 1961. Statistical estimations in enzyme kinetics. *Biochem. J.* 80:324-332.
22. Willsky, G. R., R. L. Bennett, and M. H. Malamy. 1973. Inorganic phosphate transport in *Escherichia coli*: involvement of two genes which play a role in alkaline phosphatase regulation. *J. Bacteriol.* 113:529-539.
23. Willsky, G. R., and M. H. Malamy. 1974. The loss of the *phoS* periplasmic protein leads to a change in the specificity of a constitutive inorganic phosphate transport system in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* 60:226-233.
24. Willsky, G. R., and M. H. Malamy. 1976. Control of the synthesis of alkaline phosphatase and the phosphate-binding protein in *Escherichia coli*. *J. Bacteriol.* 127:595-609.