JOURNAL OF BACTERIOLOGY, Jan. 1982, p. 123-130
0021-9193/82/010123-08$02.00/0

Vol. 149, No. 1

Phosphate Exchange in the Pit Transport System in *Escherichia coli*

H. ROSENBERG,* LESLEY M. RUSSELL,† P. A. JACOMB, AND KAYE CHEGWIDDEN

Department of Biochemistry, John Curtin School of Medical Research, Australian National University, Canberra City, A.C.T., 2601 Australia

Received 4 March 1981/Accepted 25 August 1981

The Pit system of phosphate transport in *Escherichia coli* catalyzes a rapid exchange between the external inorganic phosphate and internal phosphate pools, including some ester phosphates which are in rapid equilibrium with the internal P_i pool. Unlike net energized uptake, the P_i exchange proceeds in energy-depleted cells in the presence of uncouplers and is not accompanied by the movement of potassium ions. In the absence of externally added phosphate, the exit of P_i from the cells is insignificant. The apparent K_m for external P_i in the exchange reaction is about 7 mM (2 orders of magnitude higher than that of energized uptake), but the maximal velocity is about the same. The exchange is temperature sensitive and is affected by thiol reagents. The combined observations suggest the operation of a facilitator which is part of the Pit system. The exchange is repressed in cells grown on glucose and other phosphotransferase system substrates, but not in cells grown on other carbohydrate sources. The repression can be reversed by the addition of cyclic AMP to the medium.

Escherichia coli possess two major systems for the transport of P_i. These were originally described by Willsky et al. (23) and were designated Pit (for P_i transport) and Pst (for phosphate-specific transport). Rosenberg et al. (14) and Willsky and Malamy (24) described these systems in more detail after studying them in mutants carrying only one or the other of the two. In their study, Rosenberg et al. (14) showed that only the Pit system was capable of carrying out the exchange of P_i between the intracellular and extracellular pools. The exchange was observed in whole cells and in spheroplasts and was absent from cells of the *pit* genotype. The present study of some of the properties of this exchange reaction was undertaken to gain some further information about the nature and operation of the Pit system.

MATERIALS AND METHODS

Chemicals were of the highest purity commercially available. Triethanolamine was from Fluka. Carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) and *N*-ethylmaleimide (NEM) were from Calbiochem (Australia) Ltd., Carlingford, NSW. Carrier-free $^{32}P_i$ and ^{42}KCl were obtained from the Australian Atomic Energy Commission, Lucas Heights, NSW. Polyvinylpolypyrrolidone was from Sigma and was acid washed and dried as described by Ohnishi (7).

Bacterial strains. The strains of *E. coli* K-12 used in this study, AN710 (*phoT101 argH entA*) and AN1088 (*pit argH entA*), were described previously (14, 16). Each strain possesses only one of the two major phosphate transport systems (Pit and Pst, respectively).

Growth of cells. The growth medium and essential supplements used were as described (14). Unless otherwise specified, the carbon source for growth was 20 mM K-DL-lactate. When lactate is described as the energy source in various experiments, the concentration shown always refers to DL-lactate. The P_i-free uptake medium contained 0.05 M triethanolamine hydrochloride, 0.015 M KCl, 0.01 M $(NH_4)_2SO_4$, and 0.001 M $MgSO_4$ (pH 6.5). Stationary-phase cells were harvested after overnight growth at 37°C with shaking.

Cells were collected by centrifugation, washed twice, suspended in uptake medium at an absorbancy at 660 nm of 0.35, and shaken at 37°C for 1 h in the presence of 2 mM lactate to deplete them of phosphate (the absorbancy at 660 nm increased to about 0.39 to 0.40 during this period). The cells were then washed three times to remove the carbon source, resuspended in uptake medium at an absorbancy at 660 nm of 0.35 and stored at 3°C until required for uptake assays, but no longer than 4 h.

Depletion of energy source. In some instances the washed, phosphate-depleted cells were shaken for 1 h at 37°C with 2 mM 2,4-dinitrophenol without an energy source. The cells were washed and resuspended in uptake medium as described above.

Uptake assays. The techniques and apparatus used for the uptake assays were described elsewhere (12, 13). Unless specified in the text, assays were performed at pH 6.5 and in the absence of an added energy source. Uptake or exchange was expressed in terms of nanomoles per milligram of dry weight, by using the experimentally derived factor that an absor-

† Current address: Department of Microbiology, Uniformed Services University of Health Services, Bethesda, MD 20014.

bancy at 660 nm of 1.0 is equal to 0.43 mg (dry weight) per ml.

Assay of ^{32}P esterification. The method used for assaying ^{32}P esterification was that of Ohnishi (7), with the following modifications. The microcolumn was a disposable pipette tip with a total holding volume of 0.5 ml. It was plugged with cotton, and the bed was made with 0.2 ml of a 10% suspension of polyvinylpolypyrrolidone. The column was pretreated, and the samples were applied and washed through as originally described (7), except that the total sample volume was 0.2 ml and a wash of 0.2 ml followed. The elution was with two 0.2-ml portions of 1 N NH_4OH. The column was suitably supported to allow collection of the effluent and eluates into separate microfuge tubes of 0.5-ml capacity, which were then capped and placed in empty scintillation vials. The radioactivity was determined by Cerenkov radiation in a Packard Tri-Carb 460D counter, with channel settings 2 through 25 on the scale of 2,000. The vials were reused. Tests with standard mixtures of $^{32}P_i$ and [^{32}P]mannitol-1-phosphate showed that cross-contamination was less than 0.02% and recoveries were near 100% for both P_i and the ester. The capacity of the miniature column was found to be over 20 nmol of P_i.

RESULTS

Demonstration of the exchange reaction. As shown previously (14) the loss of intracellular ^{32}P was observed when cells which had taken up $^{32}P_i$ from a 50 μM solution for several minutes were treated with a large excess (20 mM) of unlabeled P_i. Alternatively, a cell suspension was equilibrated with 50 μM unlabeled P_i, at which point a negligible quantity (10^{-11} M) of carrier-free $^{32}P_i$ was added. The cells became rapidly labeled (Fig. 1). These results demonstrate that, at what appears to be a steady state with respect to phosphate equilibration, bidirectional movement of this anion across the membrane persists at a marked rate.

Requirement of an energy source. The exchange shown in Fig. 1 occurred in lactate-grown, washed cells with no energy source provided. When such cells were further depleted of energy by shaking at 37°C in the presence of 2 mM 2,4-dinitrophenol, the exchange still occurred, although at a lower rate; exchange was also observed when 40 μM CCCP was present. Once ^{32}P had equilibrated, further entry of radioactivity, representing net uptake, could only be elicited in the energy-depleted cells by the addition of an energy source (in this case, lactate). However, lactate prompted no uptake in the presence of CCCP (Fig. 2). Therefore, phosphate exchange can proceed under conditions where net uptake could not be seen. The absence of exchange from cells of the *pit* genotype (strain K-10) was shown previously (14). Strain K-10, however, was not isogenic with the *pit*$^+$ *phoT* strain AN710. We have compared the exchange in the isogenic pair (AN710 and AN1088) under the present experimental conditions (Fig. 3) and confirmed the observation that the *pit*$^+$ genotype is necessary for exchange to occur.

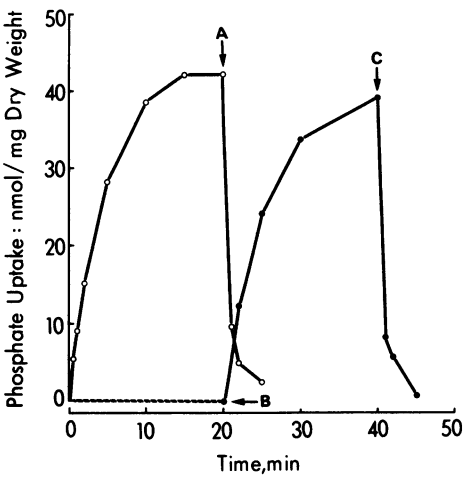

FIG. 1. Phosphate exchange in the Pit system in the absence of an added energy source. Cells were shaken with 50 μM $^{32}P_i$ for 20 min (○) when 20 mM unlabeled P_i was added (arrow A). In the second experiment (●) the cells were shaken with 50 μM unlabeled P_i for 20 min (---), and at that point (arrow B) carrier-free $^{32}P_i$ (1.4×10^{-11} M) was added to a specific radioactivity equivalent to that in the first experiment (2.5 mCi/mmol). After 20 more min, unlabeled 20 mM P_i was added to these cells (arrow C).

The effect of temperature. At temperatures near 0°C, *E. coli* fail to take up phosphate, both in the absence and in the presence of an energy source (data not shown). When cells were allowed to equilibrate with 50 μM $^{32}P_i$ at 37°C and were then cooled gradually to 0°C, the ^{32}P was retained in the cells, but the addition of 20 mM unlabeled P_i did not cause loss of label at this stage; normal exchange took place only when the cells were warmed to 37°C. When cells were cooled rapidly to 0°C, the accumulated $^{32}P_i$ was lost and did not reenter on warming, indicating that some damage to the system had taken place (data not shown).

Downhill exit of phosphate from *E. coli*. Since exchange must involve both entry and exit of phosphate, we attempted to demonstrate the exit of phosphate independently of entry. Calculation of distribution in cells at the steady state (Fig. 1) shows that even without an added energy source, the cells maintain a concentration gradient of P_i of the order of 200 to 500. Cells which had been allowed to equilibrate with $^{32}P_i$ were washed and suspended in phosphate-free medium. The exit on dilution was slight in comparison with the exchange against

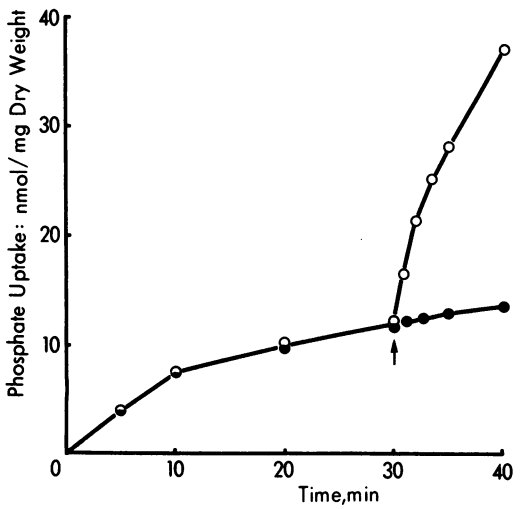

FIG. 2. Effect of CCCP on phosphate exchange and uptake in energy-depleted cells. Two suspensions of energy-depleted cells were allowed to equilibrate at 37°C with 50 μM unlabeled P_i. At this point (zero time) the control flask (○) received carrier-free $^{32}P_i$, while the other flask (●) received $^{32}P_i$ and 40 μM CCCP. After 30 min, each flask received 10 mM DL-lactate (arrow).

20 mM unlabeled P_i added to the medium (Fig. 4).

Effect of external phosphate concentration on the rate of phosphate exchange. We determined the kinetic constant for the exchange with respect to the concentration of phosphate in the medium. Energy-depleted, lactate-grown cells were allowed to exchange $^{32}P_i$ for 10 min and were centrifuged and suspended in fresh uptake medium. Unlabeled P_i was added at different concentrations to portions of this suspension, and the rate of loss of ^{32}P from the cells was measured over 2 min and calculated relative to the initial intracellular radioactivity. Reciprocal plots of exchange rates versus phosphate concentration (not shown) were drawn by computer by the method of Wilkinson (22). Four independent experiments yielded a mean value for the apparent K_m of 6.8 ± 4.3 (standard error) mM. This is about 2 orders of magnitude higher than the K_m for energized uptake in this strain (14). The value for the maximal velocity for the exchange was calculated to be 48 nmol of P per min per mg of dry weight, which was of the same order as the corresponding value of 60 found earlier for energized transport (14).

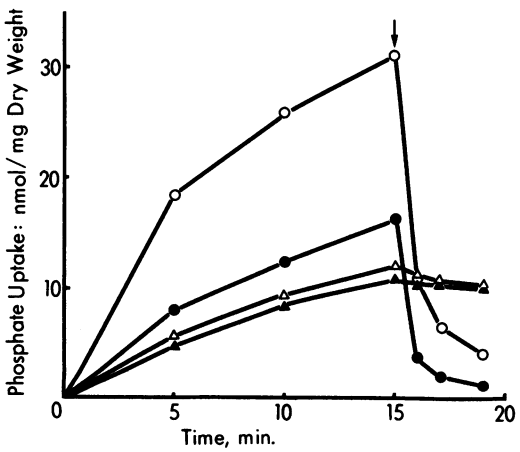

FIG. 3. Dependence of phosphate exchange on the presence of the Pit system. Cells of strain AN710, *pit*$^+$ *phoT* (●, ○), and AN1088, *pit phoT*$^+$ (▲, △), were phosphate depleted (○, △) or phosphate and energy depleted (●, ▲) and shaken with 50 μM $^{32}P_i$ for 15 min, when (arrow) nonradioactive phosphate was added to 20 mM. The suspensions were sampled at the times shown.

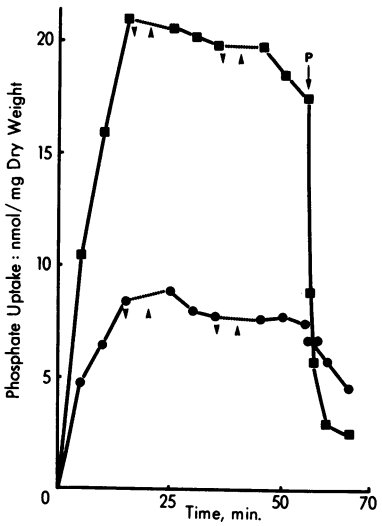

FIG. 4. Phosphate exit and exchange in the Pit system. Glucose-grown (●) and lactate-grown (■) phosphate-depleted cells were shaken with 50 μM $^{32}P_i$ for 15 min, centrifuged (▼), washed four times with uptake medium, and resuspended (▲) at the original absorbancy at 660 nm (0.35). Cell ^{32}P was monitored for 15 min, and the cells were centrifuged and resuspended in fresh uptake medium. Cell ^{32}P was monitored for a further 15 min, and at the point shown by the arrow (P) 20 mM unlabeled P_i was added to each suspension.

Effect of pH on phosphate exchange. The rate of exchange increased with increasing pH between 5.5 and 7.5, and the values followed quite closely the theoretical dissociation curve of the second proton on the phosphate (Fig. 5). It follows that HPO_4^{2-} is the actual ion participating in exchange.

Involvement of cations in phosphate exchange.

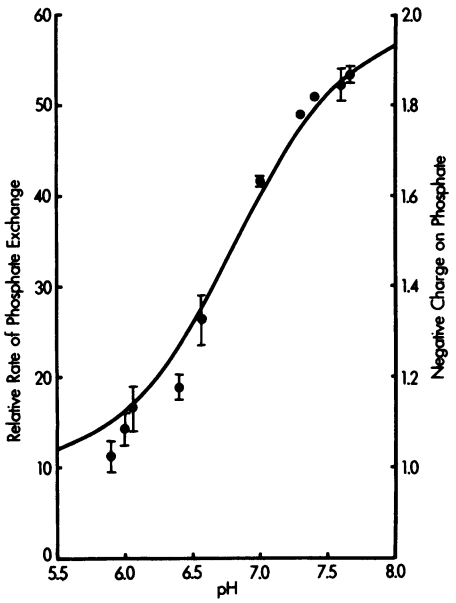

FIG. 5. Effect of pH on phosphate exchange. Energy-depleted cells were suspended in a modified uptake medium in which the 50 mM triethanolamine buffer was replaced by 2.5 mM morpholineethanesulfonic acid, and the KCl concentration was raised accordingly to maintain ionic strength. The pH remained at 6.5. Samples of the cell suspension were warmed up, 50 μM $^{32}P_i$ was added, and uptake was monitored for 2 min. At that point unlabeled P_i (20 mM final), buffered with morpholine-ethanesulfonic acid (5 mM final), was added over a range of pH. The flasks were sampled over the next 2 min, and the actual pH of the suspension was measured. The exchange rate was expressed as percentage of the ^{32}P present at 2 min which had exchanged out in the subsequent 2 min. The data are shown as means with standard error bars. Between three and six determinations were made for each point shown. Where no bars are shown, the point represents a single result at that pH. The curve drawn is the theoretical dissociation curve of the second proton of phosphate, around a pK_a of 6.82 derived by the method of Perrin and Dempsey (9) by correcting for ionic strength of the medium.

We showed previously (16, 17) that K^+ ion movement accompanies the transport of phosphate into *E. coli*. To test whether K^+ was involved in phosphate exchange, we monitored the movement of ^{42}K under conditions of exchange (Fig. 6). The addition of 20 mM (Li^+ or Na^+)P_i caused the loss of ^{32}P label from the cells, as expected, but loss of ^{42}K only occurred when the P_i was added as the Na salt, but not as the Li salt. (We found earlier [16] that Li^+ had no effect on K^+ movements.) Overall Na^+-K^+ counterflow in *E. coli* has been described by Schultz et al. (21). In another experiment we found that phosphate exchange on the addition of 20 mM P_i proceeded in the usual manner in uptake medium where K^+ was wholly substituted by Li^+ or choline (data not shown).

Effect of thiol inhibitors. NEM had no effect on phosphate exchange in energy-depleted cells, both in the test when the inhibitor (10 mM) was added to cells which had equilibrated with 50 μM $^{32}P_i$ and just before the addition of 20 mM P_i and when added to cells equilibrated with 50 μM $^{31}P_i$, just before the addition of carrier-free $^{32}P_i$. However, NEM, like CCCP, inhibited energized uptake. Mercaptoethanol did not reverse the inhibitory effect of NEM (Fig. 7). The effect of mercuric ion (Fig. 8) was distinctly different from that of NEM. Mercuric ions added at any stage arrested phosphate uptake and totally inhibited phosphate exchange which normally follows the addition of 20 mM unlabeled phosphate. Unlike the inhibition by NEM, the effect

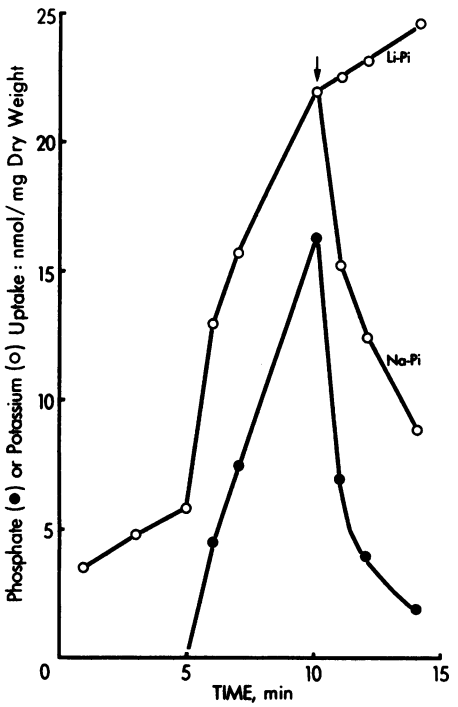

FIG. 6. Potassium ion movements under conditions of phosphate exchange. Washed, lactate-grown cells, with 20 mM glucose as an energy source, were incubated in two separate flasks in uptake medium in which K^+ ion was replaced by choline. At the start of the experiment, 1 mM ^{42}KCl (6.5 μCi/mmol) was added to flask A (○). At 5 min 50 μM nonradioactive sodium phosphate was added to flask A, and 50 μM $^{32}P_i$ was added to flask B (●). At 10 min (arrow) the contents of flasks A and B were divided, and 20 mM unlabeled Li-P_i or Na-P_i was added to one half, respectively. ^{42}K (○) and ^{32}P (●) were monitored in the appropriate suspensions. (The descending portion of the latter curve was the same for both Li-P_i and Na-P_i additions.)

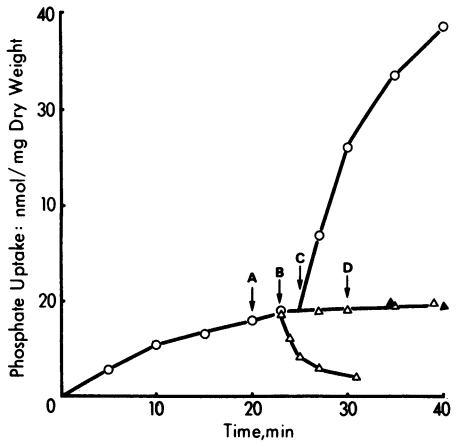

FIG. 7. Effect of NEM on phosphate exchange. Energy-depleted cells were incubated for 20 min with 50 μM $^{32}P_i$ (○). At the point shown by arrow A, 10 mM NEM was added to a portion of the cells (△), and at the point shown by arrow B 20 mM nonradioactive P_i was added to a portion of the NEM-treated cells. The control cells and the remaining NEM-treated cells, received 10 mM lactate at the point shown by arrow C, and a portion of the latter suspension received 20 mM mercaptoethanol (▲) at the point shown by arrow D. ^{32}P was monitored in all suspensions.

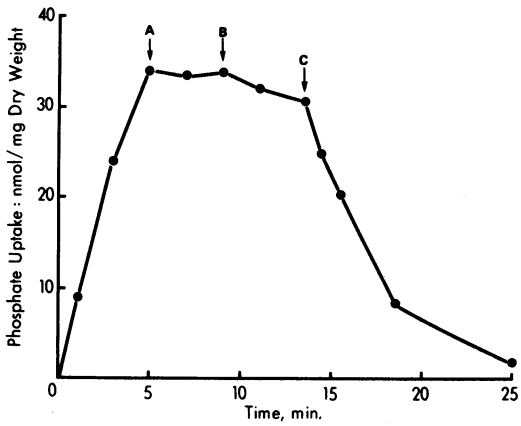

FIG. 8. Inhibition of phosphate exchange by mercuric ions and its reversal by mercaptoethanol. Thrice-washed cells were shaken with 50 μM $^{32}P_i$ in the absence of an energy source. At 5 min (arrow A), 10 μM $HgCl_2$ was added; 20 mM nonradioactive P_i was added at arrow B, and 0.4 mM mercaptoethanol at arrow C. The suspension was sampled for ^{32}P uptake at times shown (●).

of Hg^{2+} was reversed by a 40-fold excess of mercaptoethanol.

Involvement of esterified phosphate in exchange. It can be seen from Fig. 1 that over 90% of the total accumulated ^{32}P was chased from the cells when 20 mM unlabeled P_i was added to the medium. Since more than half of the intracellular ^{32}P was esterified at the time of the chase (Table 1), it follows that the ester P was also chased. Thin-layer chromatography followed by scanning of plates of cell extracts before and after chase showed that the two major labeled phosphate esters found in the extracts (see below) had lost their radioactivity (data not shown). Analysis of the ^{32}P which had exchanged into the medium showed that it was entirely (over 99%) P_i. The disappearance of radioactivity from both the ester P and P_i pools proceeded simultaneously and followed first-order kinetics (Fig. 9). In this experiment, and in two independent repeats, the half-time decay of radioactivity in the P_i and ester P fractions was about 12 and 26 s, respectively. The upward curving of the P_i line (Fig. 9) would be expected because the slower-decaying ester-phosphate pool must flow through the intracellular P_i pool before exchanging with external P_i. NEM treatment just before the chase resulted in a considerable reduction in the amount of ^{32}P chased out of the cell. An examination of the distribution of ^{32}P in the cells before and after the chase revealed that NEM had no effect on the exchange of P_i, but inhibited the chase of ^{32}P from the ester P pool (Table 1). Subsequent large-scale fractionation of extracts of ^{32}P-labeled cells showed that, of all the acid-soluble esterified ^{32}P, over 80% was distributed in two compounds, which have been identified as fructose-1,6-*bis*-phosphate and mannitol-1-phosphate (H. Rosenberg, unpublished data). The specific radioactivities of the ^{32}P in these compounds rela-

TABLE 1. Effect of NEM on ^{32}P chase from P_i and ester phosphate[a]

Expt	Total label taken up (%)		
	P_i	Ester P	Acid insoluble
1			
Before chase	27	61	12
After 5-min chase	1.5	13	12
2			
Before chase	24	64	12
After 5-min chase in presence of NEM	1.8	52	12

[a] Phosphate-depleted cells were shaken 5 min with 50 μM $^{32}P_i$ in the presence of 10 mM lactate. The suspension was divided in two; one half received 20 mM unlabeled P_i, and the other received 10 mM NEM and then 20 mM P_i 1 min later. At 5 min after the chase, the cells from each suspension were collected on membrane filters, washed, and extracted in 0.5 M $HClO_4$ at 0°C. P_i and esterified P were assayed in the extracts as described in methods. Label in the acid-insoluble fraction was calculated by difference.

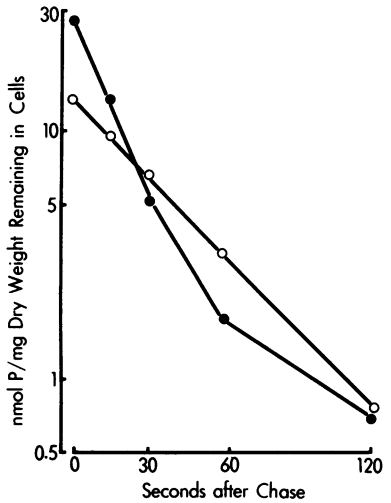

FIG. 9. Chase of ^{32}P from P_i and ester P. P-depleted cells were shaken for 5 min in uptake medium containing 10 mM K lactate and 50 μM $^{32}P_i$. At this time (0) 20 mM nonradioactive P_i was added. At each time shown, 0.5 and 2.0 ml of the suspension were independently passed through a membrane filter and then washed. The membrane with the smaller sample was dried and counted as usual. The membrane with the larger sample was extracted for 20 min (with shaking) in 1.0 ml of 0.25 M $HClO_4$ at 0°C. The extract was centrifuged to remove dislodged cells, and duplicate samples of the supernatant were used as described in the text to determine the proportions of P_i and ester P. The actual amount of each fraction was then calculated from the known total cell ^{32}P, after correcting for acid-insoluble and nonexchangeable material. The corrected calculated values of P_i (●) and ester P (○) are plotted on an exponential scale against time of chase.

tive to cell P_i (100%) were 79 and 96%, respectively, after 5 min of incubation with $^{32}P_i$. The specific radioactivity of cell $^{32}P_i$ was 91% of that in the medium.

Carbon source-mediated repression of phosphate exchange through the Pit system. As reported previously (14), the P exchange through the Pit system was observed in cells grown on succinate or lactate as carbon source, but not in glucose-grown cells. The reason for this was not understood, but the phenomenon suggested some connection to the phosphoenolpyruvate phosphotransferase system. A wider examination of the effect of various carbon sources supplemented during growth on the subsequent ability of strain AN710 to carry out phosphate exchange showed that certain other phosphotransferase system sugars repressed the exchange. The addition of cyclic AMP (cAMP) to the medium alleviated this repression to a varying extent (Table 2).

DISCUSSION

The present results indicate that the exchange of phosphate across the membrane of *E. coli* is a facilitated process depending on the *pit* product. Strain AN1088, carrying the *pit* mutant allele, lacks the exchange, whereas strain AN710, carrying the *phoT* mutant allele (but with the Pit system intact), shows normal exchange, as do also wild strains with both the Pit and Pst systems intact (14). The exchange observed in strain AN710 (*pit*$^+$ *phoT*) is therefore not due to a defect in the Pst system, but reflects the presence of a functional Pit system. The present results show that the exchange proceeds under conditions of energy depletion. It should be stressed that these cells are not totally depleted of energy. However, such cells cannot carry out net phosphate uptake unless an energy source is supplied (Fig. 2).

It is not clear what advantage (if any) the cells derive from the rapid bidirectional flow of phosphate at the steady state or at any other time. We have shown (16, 17) that the movements of both protons and K^+ ions are intimately linked with the transport of phosphate in *E. coli*, and we have checked the possible involvement of phosphate in some steady-state circulation of

TABLE 2. Effect of carbon source and cAMP supplied during growth on subsequent phosphate exchange in *E. coli*[a]

Carbon source	cAMP (mM)	% Exchange in 1 min
Glucose	0	5.0 ± 1.9 (3)
	2.0	18.2 ± 0.8 (3)
Fructose	0	12.1 ± 3.4 (4)
	2.0	48.0 ± 3.1 (4)
Mannitol	0	12.1 ± 1.8 (6)
	2.0	40.8 ± 5.5 (6)
Melibiose	0	29.1 ± 3.7 (3)
	2.0	47.5 ± 10.6 (3)
Lactose	0	28.5 ± 5.3 (4)
	2.0	44.3 ± 7.4 (5)
Lactate	0	53.3 ± 0.9 (4)
Succinate	0	66.7 ± 1.9 (4)

[a] Cells were grown overnight with or without 2 mM cAMP on carbon sources as shown (20 mM for sugars, 25 mM for carboxylic acids). They were washed and resuspended in uptake medium containing the same carbon source as used for growth, but at 10 mM. The cells were shaken for 20 min at 37°C to deplete traces of phosphate from the medium, 50 μM $^{32}P_i$ was added, and uptake was monitored for 2 min. At 2 min 20 mM unlabeled P_i was added, and loss of radioactivity was monitored for 1 min. The data were calculated as percentage of ^{32}P lost from the cells during that minute. Means of several experiments (number shown within parentheses), with the standard errors of the mean, are presented.

ions, but phosphate exchange was found to proceed without K^+ ion movement (Fig. 6).

A mechanistic model that best fits these and previous (13, 15) observations would conform generally with the chemiosmotic interpretation of Mitchell (6) and the originally observed movements of protons with the phosphate ion (5). We have also shown (16) that, depending on external pH, up to three protons enter the cells with each phosphate ion and that this is immediately followed by the inward movement of K^+ ions which is not directly coupled to any particular phosphate system and is interpreted as a K^+-H^+ exchange aimed at maintaining the internal pH (17). The active transport of phosphate may involve a net entry of positive charge, similar to the entry of a positively charged complex of phosphate and three protons in yeast observed by Cockburn et al. (1).

In our interpretation of the exchange mechanism through the Pit system, we favor the proposition that the K_m of the carrier for substrate can alter, depending on the state of energization (3, 19, 20, 25). In the Pit system, which is energized by proton motive force (13, 15), the occupancy of a specific site(s) by proton(s) could result in the low K_m observed (26 μM) in the outward orientation of the P_i site. The K_m on the inward orientation would be high—in the 0.01 M range. Such an arrangement would ensure the saturation of the carrier on the outside (with P_i at 50 μM) and release of P_i on the inside, at least until the internal concentration found in normal cells (20 to 30 mM) is reached. At this stage, reciprocal P_i movement occurs, and this explains the exchange seen in the presence of substrate.

When energy is insufficient for active transport, the K_m of the outward-oriented and inward-oriented carriers is approximately equal and in the 0.01 M range, the value we find for the exchange process. If P_i in such concentration is present on the outside (as it is on the inside), the carrier is equally saturated in both orientations and exchange occurs. It is likely that a limited amount of energy is required for the process. To explain the lack of all but slight downhill exit when external phosphate is low or near zero, we hypothesize that the carrier cannot reorient from either conformation unless a molecule of P_i is bound. Thus, under the conditions of energy depletion, once cells are resuspended in phosphate-free medium, the carrier can still bind phosphate on the inside and reorient. In the outward conformation, the phosphate dissociates, and the carrier cannot return to the previous orientation, since at the high K_m it cannot bind P_i on the outside. In this respect the system differs from others, such as the lactose and galactose transporters, which show downhill exit. The present hypothesis envisages that the carrier exhibits the high K_m with no protons bound. The HPO_4^{2-} ion therefore exchanges in a non-electrogenic fashion, and, since no net acidification of the interior takes place, the movement of K^+ ions need not occur during exchange. This is supported by the data in Fig. 6.

Several observations support the proposition that both the exchange and transport are mediated by the Pit system. Thus, both processes are reversibly inactivated at low temperatures, and both are blocked by Hg^{2+}, which has been used as a stopping reagent with the lactose carrier (4). Furthermore, the exchange process cannot be resolved into independent entry and exit reactions, as seen from the failure to induce downhill exit by dilution (Fig. 4).

Since active phosphate transport in the Pit system is supported by proton motive force (15), its rate increases with decreasing pH in the range 7.5 to 6.0 as long as the amount of phosphate present is sufficient to maintain adequate concentrations of the transported ion, HPO_4^{2-} (H. Rosenberg, unpublished observation). On the other hand, the exchange process was found to be independent of change in pH: it proceeded in the presence of CCCP, and, with 20 mM P_i present, its rate increased with pH and followed closely the dissociation curve of the second proton of phosphate, indicating that, like the active process, it also involved the HPO_4^{2-} ion. When data from experiments shown in Fig. 5 were replotted in inverse fashion against the derived true concentration of HPO_4^{2-} at the given pH values, a K_m value in the range of 2 to 4 mM was obtained. This compares well with the K_m value of about 7 mM quoted above. That value was derived at pH 6.5 and obtained by inverse plot using the concentration of total P_i added. Since at pH 6.5 only about one-third of the total P_i is in the form of HPO_4^{2-}, the comparison of the two K_m values confirms that this ion is the one involved in exchange.

Phosphate entering the cell was found to be rapidly esterified under normal energized conditions. This process was inhibited by NEM. The rapid equilibrium between the ester P and P_i pools resulted in both pools exchanging with external phosphate. NEM was shown to prevent the participation of the ester pool phosphate in the exchange, thus explaining its depression of the overall extent of the exchange. The effect on the actual exchange of P_i itself was minimal. Thus, NEM, unlike Hg^{2+} ions, had no effect on the carrier, but rather affected some phosphoryl transfer reactions in the cell.

We reported previously (14) that the exchange reaction in the Pit system was repressed in cells grown on glucose. The present results expanded this observation to show that other sugars (fruc-

tose and mannitol), via the phosphotransferase system (18), also repressed the exchange. Cells grown on non-phosphotransferase sugars (melibiose and lactose) showed good exchange, although not as extensive as those grown on the carboxylic acids. Like other phosphotransferase-repressed processes (8), the phosphate exchange was derepressed by 2 mM cAMP added to the medium. These results point to the interaction of the phosphotransferase system with some component of the Pit system, probably indirectly by its repression of cAMP levels and adenylate cyclase, and of the state of phosphorylation of hypothetical regulatory proteins (2, 10, 11). It is not possible at present to offer any suggestions about the nature of these interactions.

ACKNOWLEDGMENTS

We thank J. F. Morrison for his advice and assistance with the kinetic work and H. C. F. Dixon for helpful discussions on the coupled decay of radioactivity in the inorganic and ester-P fractions. Jane Jones provided excellent technical assistance.

The award of an Australian National University Scholarship to L.M.R. is acknowledged.

LITERATURE CITED

1. **Cockburn, M., P. Earnshaw, and A. A. Eddy.** 1975. The stoicheiometry of the absorption of protons with phosphate and L-glutamate by yeasts of the genus Saccharomyces. Biochem. J. **146**:705–712.
2. **Feucht, B. U., and M. H. Saier, Jr.** 1980. Fine control of adenylate cyclase by the phosphoenolpyruvate: sugar phosphotransferase systems in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **141**:603–610.
3. **Hill, T. L.** 1969. A proposed common allosteric mechanism for active transport, muscle contraction and ribosomal translocation. Proc. Natl. Acad. Sci. U.S.A. **64**:267–274.
4. **Lancaster, J. R., and P. C. Hinkle.** 1977. Studies of the β-galactoside transporter in inverted membrane vesicles of *Escherichia coli*. I. Symmetrical facilitated diffusion and proton gradient-coupled transport. J. Biol. Chem. **252**:7657–7661.
5. **Mitchell, P.** 1954. Transport of phosphate across the osmotic barrier of *Micrococcus pyogenes*: specificity and kinetics. J. Gen. Microbiol. **11**:73–82.
6. **Mitchell, P.** 1973. Performance and conservation of osmotic work by proton-coupled solute porter systems. J. Bioenerg. **4**:63–91.
7. **Ohnishi, S. T.** 1978. A new method of separating inorganic orthophosphate from phosphoric esters and anhydrides by an immobilized catalyst column. Anal. Biochem. **86**:201–213.
8. **Pastan, I., and S. Adhya.** 1976. Cyclic adenosine 5'-monophosphate in *Escherichia coli*. Bacteriol. Rev. **40**:527–551.
9. **Perrin, D. D., and B. Dempsey.** 1974. Buffers for pH and metal ion control, p. 6–20. Chapman & Hall, Ltd., London.
10. **Peterkofsky, A., and C. Gazdar.** 1978. The *Escherichia coli* adenylate cyclase complex: activation by phosphoenolpyruvate. J. Supramol. Struct. **9**:219–230.
11. **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.
12. **Rosenberg, H.** 1979. Transport of iron into bacterial cells. Methods Enzymol. **56**:388–394.
13. **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.
14. **Rosenberg, H., R. G. Gerdes, and K. Chegwidden.** 1977. Two systems for the uptake of phosphate in *Escherichia coli*. J. Bacteriol. **131**:505–511.
15. **Rosenberg, H., R. G. Gerdes, and F. M. Harold.** 1978. Energy coupling to the transport of inorganic phosphate in *Escherichia coli* K12. Biochem. J. **178**:133–137.
16. **Russell, L. M., and H. Rosenberg.** 1979. Linked transport of phosphate, potassium ions and protons in *Escherichia coli*. Biochem. J. **184**:13–21.
17. **Russell, L. M., and H. Rosenberg.** 1980. The nature of the link between potassium transport and phosphate transport in *Escherichia coli*. Biochem J. **188**:715–723.
18. **Saier, M. H., Jr.** 1977. Bacterial phosphoenolpyruvate:sugar phosphotransferase systems: structural, functional and evolutionary interrelationships. Bacteriol. Rev. **41**:856–871.
19. **Schachter, D., N. Johnson, and M. A. Kirkpatrick.** 1966. Energy coupling for galactoside accumulation in *Escherichia coli*. Biochem. Biophys. Res. Comm. **25**:603–608.
20. **Schachter, D., and A. Mindlin.** 1969. Dual influx model of thiogalactoside accumulation in *Escherichia coli*. J. Biol. Chem. **244**:1808–1816.
21. **Schultz, S. G., W. Epstein, and A. K. Solomon.** 1963. Cation transport in *Escherichia coli*. IV. Kinetics of net K uptake. J. Gen. Physiol. **47**:329–346.
22. **Wilkinson, G. N.** 1961. Statistical estimations in enzyme kinetics. Biochem. J. **80**:324–332.
23. **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.
24. **Willsky, G. R., and M. H. Malamy.** 1980. Characterization of two genetically separable inorganic phosphate transport systems in *Escherichia coli*. J. Bacteriol. **144**:356–365.
25. **Wong, J. T.-F., A. Pincock, and P. M. Bronskill.** 1971. Site of energy coupling in the carrier mechanism for β-galactoside transport. Biochim. Biophys. Acta **233**:176–188.