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Effect of Silver Ions on Transport and Retention of Phosphate by *Escherichia coli*

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Silver ions inhibited phosphate uptake and exchange in *Escherichia coli* and caused efflux of accumulated phosphate as well as of mannitol, succinate, glutamine, and proline. The effects of Ag^+ were reversed by thiols and, to a lesser extent, by bromide. In the presence of *N*-ethylmaleimide and several uncouplers, Ag^+ failed to cause phosphate efflux, but still inhibited exchange of intracellular and extracellular phosphate, indicating an interaction at more than one site. It is unlikely that Ag^+ caused metabolite efflux by acting solely as an uncoupler, as an inhibitor of the respiratory chain, or as a thiol reagent.

Silver ions inhibit the respiratory chain of *Escherichia coli* (29), possibly at two sites of different sensitivity (3), and were also reported to exert an uncoupler-like action (4). Inhibition by silver ions of succinate uptake into membrane vesicles of *E. coli* has been noted (17), and its inhibition of NADP-linked glutamate dehydrogenase prompted the suggestion that sulfhydryl groups might be involved (8).

Like silver, zinc ions also inhibit the respiratory chain, possibly at the less sensitive site of silver inhibition (3), and almost completely inhibit the uptake of galactose and leucine into *E. coli* (1). There is no effect on the binding of galactose and leucine, so a process related to energy coupling may be affected. Again, it is possible that sulfhydryl groups are involved (9).

Trialkyltin compounds have been shown to catalyze Cl^--OH^- exchange across mitochondrial membranes in media containing chloride (24, 26). Singh and Bragg (25) observed an inhibition by tributyltin chloride of the uptake of proline and glutamine by *E. coli*. The inhibitor also caused an efflux of previously accumulated proline and glutamine. They concluded that the OH^--anion exchange catalyzed by tributyltin chloride dissipated the ΔpH component of the proton motive force and was responsible for the action of the inhibitor on proline and glutamine uptake and retention.

We have investigated the effects of silver ions on the accumulation of P_i in *E. coli*, where there are two major systems, Pit and Pst (27), for its transport. These systems have been found to differ in the nature of their energy coupling (20, 21). The Pit system is coupled to the proton motive force (12), and the Pst system appears to be energized by phosphate bond energy (2). It was therefore of interest to observe the effects of silver ions on these two systems.

In this paper we show that silver ions inhibited the uptake of P_i and, when added to cells which had taken up P_i, caused the efflux of the accumulated phosphate. The exchange of intracellular and extracellular phosphate (20) was also inhibited.

MATERIALS AND METHODS

Tributyltin chloride was obtained from Tokyo Kasei, Tokyo, Japan, and converted to tributyltin acetate by passing it through Dowex 1 in the acetate form. Carrier-free $^{32}P_i$ was from the Australian Atomic Energy Commission, Lucas Heights, NSW, Australia. [*U*-^{14}C]proline, [*U*-^{14}C]glutamine, [1-^{14}C]mannitol and [2,3-^{14}C]succinate were from the Radiochemical Centre, Amersham, United Kingdom.

Bacterial strains. The strains of *E. coli* K-12 used in this work were AN710 (*argH entA phoT101*), which contains only the Pit system for the transport of P_i (20), and AN1088 (*argH entA pit-1*), containing the Pst system (23). These strains were otherwise isogenic. The phosphate-negative strain E15 (*pit-1 phoA-8*) of Fan et al. (7) was used in one experiment.

Growth media and buffers. The growth medium for cells has been described previously (23). This medium was supplemented with 3 μM thiamine, 10 μM 2,3-dihydroxybenzoate, 1 mM arginine, and either 20 mM glucose or 20 mM succinate. Chloride-free, phosphate-free medium used to deplete the cells of P_i and for P_i uptake measurement contained the following: 50 mM triethanolamine, 10 mM $(NH_4)_2SO_4$, 1 mM $MgSO_4$, and 15 mM K_2SO_4. The pH was adjusted to 6.5 at 37°C with citric acid. The wash solution used in the uptake assays has been described previously (20). In some cases, as mentioned below, the uptake buffer used contained 50 mM 2-(*N*-morpholino)ethanesulfonic acid, adjusted to pH 6.5 with triethanolamine base, but otherwise the same as that described above.

Preparation of cells and measurement of P_i uptake.

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 either 5 mM succinate or 1 mM glucose to deplete them of phosphate. The same energy source was used for growth and P_i depletion and uptake. During the depletion period the absorbancy at 660 nm increased to 0.39 to 0.40. The P_i-depleted cells were washed twice to remove the energy source, suspended in uptake medium containing 1 mM 2,4-dinitrophenol, and shaken for 1 h at 37°C. They were then washed three times and suspended 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. Uptake measurements were performed as described elsewhere (19, 20). Uptake or exchange was expressed as nanomoles per milligram (dry weight) of cells, by using the following experimentally derived factor: absorbancy at 660 nm of 1.0 = 0.43 mg (dry weight) per ml.

Analysis of P_i and esterified phosphate. P_i and esterified phosphate were assayed by a modification (22) of the method of Ohnishi (15).

Assay of cell viability. After cells were treated as described below, they were diluted and plated onto solid medium containing the following: 1% tryptone, 1% beef extract, 0.5% NaCl, 0.3% yeast extract, 2% agar, and 2 mM glucose. Colonies were counted after overnight growth at 37°C.

RESULTS

Inhibition of P_i uptake by Ag^+. Treatment of cells with silver ions inhibited P_i transport in both the Pit and Pst systems, but to a differing extent (Table 1). The degree of inhibition did not depend upon the length of time of preincubation with $AgNO_3$ (data not shown). A higher concentration of Ag^+ was required to inhibit the Pst system. The addition of KBr to remove excess Ag^+ relieved the inhibition to some extent, but not completely. Cells grown and assayed with glucose as the sole carbon source were more resistant to the effect of Ag^+.

TABLE 1. Inhibition of phosphate uptake by silver ions[a]

Strain	Energy source[b]	KBr added (mM)	$AgNO_3$ concn (μM) to inhibit:	
			50%	95%
AN710	Succinate	0	1.2	3.7
		2	1.7	8.6
	Glucose	0	2.6	4.7
		2	3.3	12.6
AN1088	Succinate	0	3.3	50
		2	7.6	79
	Glucose	0	4.0	71
		2	12.0	96

[a] The cell suspensions were incubated at 37°C for 5 min, in the presence of the energy source (20 mM) shown, before the addition of $^{32}P_i$. In the control suspension, $^{32}P_i$ was added and uptake followed for 1 min. Silver-treated suspensions received $AgNO_3$ 2 min before $^{32}P_i$. KBr was added 1 min after the $AgNO_3$ (1 min before the $^{32}P_i$). In those suspensions $^{32}P_i$ uptake was also followed for 1 min.

[b] Cells were always grown and phosphate depleted in the same energy source as used for uptake assays.

Effect of Ag^+ on accumulated P_i. When $AgNO_3$ was added to a suspension of cells which had taken up $^{32}P_i$, an efflux of radioactivity was observed (Fig. 1). Again, the effect on the Pit

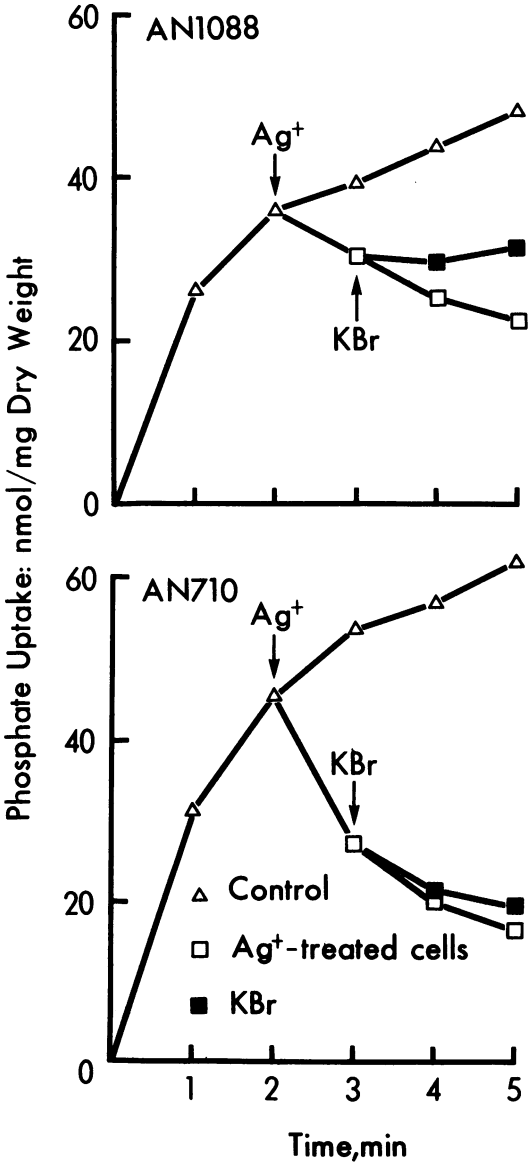

FIG. 1. Efflux of phosphate caused by Ag^+ ions. Phosphate uptake was assayed as described in the text. Additions of 20 μM $AgNO_3$ and 2 mM KBr were as shown. The source of energy was 20 mM succinate.

system was more pronounced than that on the Pst system. The addition of KBr to remove excess Ag^+ caused uptake to resume after about 5 min in cells with the Pst system (data not shown). This was never observed in cells carrying only the Pit system. The effect of Ag^+ was less pronounced when glucose, rather than succinate, was the source of energy (data not shown).

Effect of $AgNO_3$ concentration on phosphate efflux. The concentration of $AgNO_3$ required to cause net phosphate exit at a given rate varied with cell density, indicating that titration of some essential sites with Ag^+ occurred. However, a linear relationship between cell density and Ag^+ concentration required for a given effect was not maintained over the range of absorbancies at 660 nm tested, 0.18 to 0.72 (data not shown).

Nature of phosphate released by Ag^+ treatment. Phosphate is rapidly esterified after it enters the cell (11). If Ag^+ damaged the cell membrane, making it freely permeable, esterified phosphate should appear outside the cell after silver treatment. We tested this by assaying the proportion of ^{32}P in ester form in cells before and after $AgNO_3$ treatment and in the medium at the end of the treatment (Table 2). It is clear that the ^{32}P found in the medium after Ag^+ treatment is largely (96 to 97%) P_i and that a portion of it is derived from cellular ester phosphate. Control cells (not treated with Ag^+) lost less than 5% of the total ^{32}P during the experiment. Since the strain used (AN710, *phoT*) was constitutive for the periplasmic alkaline phosphatase, the possibility existed that some phosphate esters, released from the cells by Ag^+ treatment, were hydrolyzed to P_i in the periplasm. We tested this by repeating the experiment with a phosphatase-negative mutant strain, E15 (7). The result (Table 2) was similar to that observed with strain AN710. A similar result (data not shown) was obtained with spheroplasts from strain AN710.

TABLE 2. Nature of phosphate released from cells by Ag^+ treatment[a]

Strain	Temp (°C)	Sampling time	% total P within cells	% ester phosphate	
				Cells	Medium
AN710	37	30 s before Ag^+	100	57.0	ND[b]
		5 min after Ag^+	46.0	34.2	2.0
AN710	0	30 s before Ag^+	100	76.0	ND
		5 min after Ag^+	58.6	39.6	4.5
E15	37	30 s before Ag^+	100	65.4	ND
		5 min after Ag^+	51.0	34.4	6.2

[a] Cells at an absorbancy at 660 nm of 0.35 were allowed to take up $^{32}P_i$ for 5 min, and the cell suspension was centrifuged, suspended in fresh buffer at an absorbancy at 660 nm of 1.75, and shaken at 37°C. $AgNO_3$ (25 μM to AN710, 100 μM to E15) was added 3 min later. Samples (1 ml) were taken at the times shown and filtered through cellulose nitrate filters (pore size, 0.45 μm), and the filters containing the cells were extracted immediately with 0.25 M perchloric acid at 0°C and assayed for P_i and esterified phosphate as described in the text. The medium samples (filtrates) from the Ag^+-treated suspensions were acidified with perchloric acid to 0.25 M and assayed for P_i and esterified phosphate as described in the text. Results are expressed as percentages of the total ^{32}P inside the cell before $AgNO_3$ addition. After uptake of phosphate and suspension at an absorbancy at 660 nm of 1.75 as described above, cell suspensions were cooled gradually to 0°C. $AgNO_3$ was then added, and samples were taken and assayed as described above.

[b] ND, Not detected.

Viability of cells after treatment with Ag^+. To further ensure that efflux of P_i was not due to cell damage, the effect of silver ions on the viability of cells was examined. Exposure to 20 μM Ag^+ over 10 min resulted in a steady decline of viability down to 40%, but more than 80% of cells remained viable within the first 2 min of Ag^+ addition (data not shown), during which period P_i efflux normally occurs. Efflux therefore could not be due to cell lysis.

Effect of Zn^{2+} on P_i transport. Zinc ions inhibited P_i uptake at concentrations about 1,000 times higher than those of silver (data not shown). Inhibition of P_i uptake by 1 mM $ZnSO_4$ was 58% and 34% in strains AN710 and AN1088, respectively. Concentrations of Zn^{2+} above 1 mM could not be tested because zinc phosphate precipitated and was trapped on the filters used in the uptake assay.

Effect of inhibitors of the respiratory chain on accumulated phosphate. The effect of Ag^+ on *E. coli* was originally identified as the inhibition of the respiratory chain (3, 29). We therefore tested whether the efflux of phosphate could be elicited by the inhibition of the respiratory chain by other inhibitors. We tested the effect of 1 mM cyanide and of 200 μM 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide. In all cases the inhibitors, when added to cells in the course of accumulation of P_i, caused the immediate arrest of uptake, but no efflux (data not shown).

Effect of Ag^+ on other transport systems. The effect of Ag^+ on accumulated proline, glutamine, mannitol, and succinate was examined (Table 3). Silver ions caused efflux of the accumulated substrate in all cases. Proline efflux was greater than glutamine efflux under the same conditions. The rate of efflux of both proline and glutamine was reduced when glucose, rather than succinate, was the source of energy. Almost identical results were obtained in strain AN1088 (data not shown).

TABLE 3. Effect of Ag^+ on other transport systems[a]

Uptake system	Energy source[b] (20 mM)	% Efflux in 1 min
Pit	Succinate	41.3 ± 1.7 (6)
	Glucose	19.1 ± 1.1 (6)
Pst	Succinate	19.0 ± 1.3 (6)
	Glucose	13.6 ± 0.9 (6)
Proline	Succinate	90.5 ± 0.3 (4)
	Glucose	57.4 ± 2.2 (4)
Glutamine	Succinate	61.9 ± 2.2 (4)
	Glucose	32.6 ± 1.7 (4)
Mannitol	Succinate	37.7 ± 0.3 (2)
Succinate	None	42.4 ± 1.5 (2)

[a] Cells were grown and prepared for uptake as described in the text, except that cells intended for the assay of mannitol uptake were shaken with 5 mM mannitol during the phosphate depletion stage. Chloramphenicol (50 μg/ml) was added before assaying amino acid uptakes, and 1 mM P_i was added to all assays other than those of $^{32}P_i$ uptake. Substrate concentrations for uptakes were as follows: P_i (both systems) 50 μM; mannitol, 20 μM, succinate and amino acids, 10 μM. Uptake of $^{32}P_i$ was monitored as described elsewhere (19, 20). The other substrates were labeled with ^{14}C (see text), and their uptake was assayed as for $^{32}P_i$, except that the dried membrane filters were immersed in 10 ml of a scintillation cocktail of 0.5% 2,5-diphenyl-oxazole in xylene–Triton X-114 (2:1 by vol) and counted in a Packard Tricarb 460CD counter. Accumulation of each substrate was monitored for 2 min. At 2 min $AgNO_3$ (20 μM) was added, and loss of radioactivity was monitored for 1 min. The data were calculated as percentage of radioactivity lost from the cells during that minute. Means of several experiments (numbers shown in parentheses) with the standard error of the mean are presented.

[b] Energy source for both growth and uptake. Cells for succinate uptake were grown with succinate as a source of carbon.

The modification by CCCP of the effects of Ag^+. When carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP) was added to cells accumulating P_i, further uptake was blocked and Ag^+ no longer caused P_i efflux from these cells (Fig. 2A). CCCP-treated cells still showed phosphate exchange, but this was blocked by Ag^+ (Fig. 2B). Three other uncouplers tested, carbonyl-cyanide-*p*-trifluoromethoxyphenylhydrazone, tetrachlorosalicylanilide, and 2,4-dinitrophenol, all blocked P_i uptake, but did not cause P_i efflux. Carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone and tetrachlorosalicylanilide also prevented the Ag^+-mediated P_i efflux (data not shown).

Effect of *N*-ethylmaleimide on Ag^+ action. The sulfhydryl reagent *N*-ethylmaleimide has been shown to inhibit the uptake of phosphate through the Pit system without interfering with exchange (22). Under the same conditions, *N*-ethylmaleimide inhibited the Ag^+-induced efflux of P_i (Fig. 3A). In the presence of *N*-ethylmaleimide, Ag^+ blocked the exchange of internal and external P_i (Fig. 3B).

Effect of tributyltin on accumulated substrates. Tributyltin, in the presence of chloride, caused efflux of accumulated phosphate, proline, and glutamine (Table 4). In the absence of added chloride, tributyltin also caused efflux, but higher concentrations were required for the same effect. Efflux in the presence of chloride was always greater than efflux in the presence of nitrate.

Effects of thiols on efflux of phosphate caused by silver ions or tributyltin. The addition of dithiothreitol or mercaptoethanol to cells which were losing $^{32}P_i$ in response to Ag^+ or tributyltin treatment caused uptake of $^{32}P_i$ to resume (data not shown). Silver ions and tributyltin are known to interact with sulfhydryl groups, and it seems likely that the thiols are protecting the cells by combining with the inhibitors and forming an inactive complex. In tributyltin-treated cells, dithiothreitol was more effective at restoring uptake in the absence of chloride than in its presence (data not shown).

Effect of Ag^+ at low concentrations on exchange of intracellular phosphate. The addition

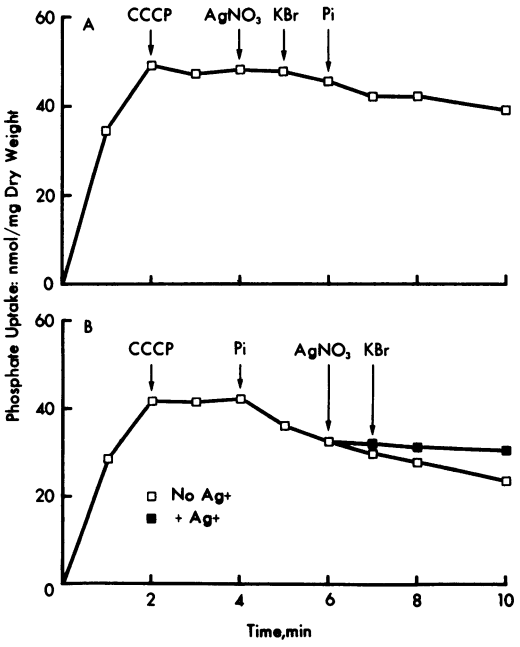

FIG. 2. Effects of CCCP and Ag^+ on phosphate exchange. Phosphate uptake was assayed in strain AN710 as described in the text. (A) Ag^+ added before 20 mM P_i. (B) Ag^+ added during exchange. CCCP (10 μM), 2 mM KBr, 20 mM P_i, and 20 μM $AgNO_3$ were added as shown.

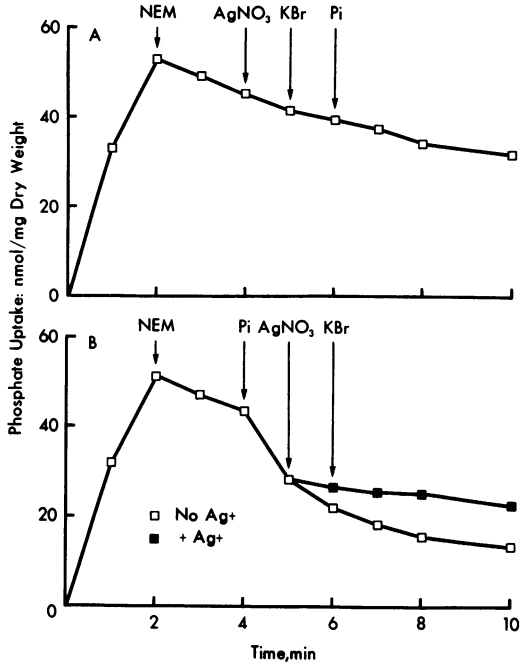

FIG. 3. Effects of *N*-ethylmaleimide and Ag^+ on exchange. Phosphate uptake was assayed in strain AN710 as described in the text. (A) Ag^+ added before 20 mM P_i. (B) Ag^+ added during exchange. *N*-Ethylmaleimide (50 μM), 2 mM KBr, 20 μM $AgNO_3$, and 20 mM P_i were added as shown.

of $AgNO_3$ at concentrations of about 2 μM caused phosphate accumulation to cease, but did not cause significant efflux of the accumulated P_i. Under these conditions the effect of adding 20 mM unlabeled phosphate was examined, and the exchange of intracellular and extracellular phosphate was found to be inhibited (Fig. 4). At an Ag^+ concentration of 1 μM, neither phosphate uptake nor phosphate exchange was affected.

Effect of temperature on the action of silver ions. At temperatures close to 0°C, *E. coli* cells do not take up phosphate and, provided the change in temperature is gradual, previously accumulated ^{32}P is retained and exchange does not occur (22). However, when cells which had accumulated ^{32}P were cooled to 0°C and then treated with 20 μM $AgNO_3$, efflux of ^{32}P still occurred, and the pattern of P_i and ester phosphate distribution was only slightly different from that observed at 37°C (Table 2).

DISCUSSION

The efflux of P_i (and of other metabolites) observed when *E. coli* was treated with $AgNO_3$ occurred in the absence of a general collapse of the permeability barrier and from viable cells,

TABLE 4. Effect of tributyltin on accumulated substrates[a]

Uptake system	Anion (20 mM)	% Efflux[b]
Pit	NO_3^-	−8.7
	Cl^-	6.0
Pst	NO_3^-	−5.3
	Cl^-	2.5
Proline	NO_3^-	5.9
	Cl^-	73.3
Glutamine	NO_3^-	34.4
	Cl^-	69.7

[a] The experiment was carried out essentially as described in footnote *a* of Table 3, except that the uptake media also contained either 20 mM KNO_3 or KCl, and 10 μM tributyltin acetate was added at 2 min. The source of energy was 20 mM succinate.

[b] A negative value indicates that uptake continued.

suggesting some form of specific exit. Previous work (22) clearly shows that, under steady-state conditions where P_i exchange takes place, entry and exit cannot be separated and exit cannot be elicited by dilution of P_i-loaded cells into P_i-free medium. The effect of Ag^+ could not therefore be explained as net exit due to selective inhibition of entry of P_i during steady state.

The most likely mechanism of this effect of Ag^+ is the collapse of the combined cellular energy resources to a level where maintenance of concentration gradients of metabolites was no longer possible. At the same time, some facility for the downhill exit of the respective metabolites should be available.

Silver ions can be expected to interact with cells at a variety of sites, the majority of which are not concerned with the effects of Ag^+ on metabolite transport. This is reflected in the

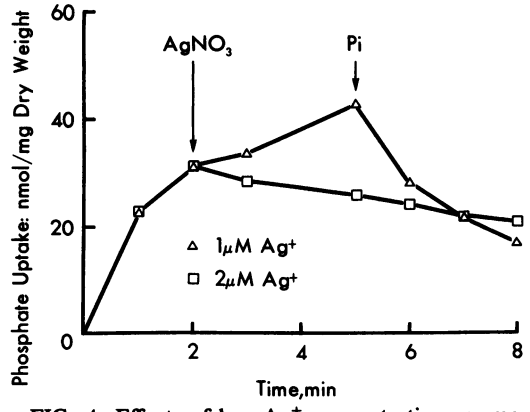

FIG. 4. Effects of low Ag^+ concentrations on uptake and exchange of P_i. Phosphate uptake was assayed in strain AN710 as described in the text. $AgNO_3$ (1 μM or 2 μM) and P_i (20 mM) were added as shown.

finding that the rate of phosphate efflux was not proportional to the Ag^+/cell ratio over the range tested.

In consideration of potential targets for the action of Ag^+ relevant to transport phenomena we singled out the following: (i) interference with energy-yielding reactions of the respiratory chain, (ii) collapse of the proton motive force, and (iii) interference with specific carriers. Any or all of these may involve -SH groups. The inhibition of the respiratory chain by Ag^+ has been documented (3, 29), and its uncoupler-like action has been reported (4). We found Ag^+ to collapse the proton motive force (Schreurs and Rosenberg, unpublished observation). Its interference with the Pit system in the presence of *N*-ethylmaleimide or CCCP reported here suggests a possible effect on the carrier.

In this paper, we eliminated each of these possibilities as the sole factor in the action of Ag^+. A number of inhibitors of the respiratory chain, while inhibiting uptake, did not elicit efflux of P_i. This was the case with cyanide, inhibiting cytochrome *d*; 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide, which inhibits near cytochrome *b* (5), where Ag^+ also acts (3); or Zn^{2+}, which is thought to inhibit between NADH or succinate and flavoprotein, at the other site for Ag^+ (3). It seems unlikely, therefore, that the effects of Ag^+ are solely due to its inhibition of the respiratory chain, nor can these effects be due to the uncoupling action of Ag^+ alone, since we showed that none of the four uncouplers tested caused P_i efflux. Among these uncouplers two (CCCP and carbonylcyanide-*p*-trifluoro methoxyphenylhydrazone) are also thiol reagents, but two others (tetrachlorosalicylanilide and 2,4-dinitrophenol) are not. The modification of the P_i carrier by Ag^+ through interaction with thiols is likely. This is supported by the fact that P_i uptake is inhibited by *N*-ethylmaleimide and by Hg^{2+} (22) and by the present finding that, under certain conditions, Ag^+ abolished both uptake and exchange of P_i. The reversal of the effects of Ag^+ by dithiothreitol also lends support to this proposal. Nevertheless, the observation that *N*-ethylmaleimide did not cause significant P_i efflux indicates that Ag^+ did not act solely as a thiol reagent in causing P_i efflux. The reversal of Ag^+ action by thiols may simply reflect a general reaction with Ag^+ which would serve to remove it from any complex, not necessarily one with another thiol. One such complex in which Ag^+ is highly specific is AgCl. To test the hypothesis that the specific effect of Ag^+ (causing P_i efflux) involved its interaction with chloride, we investigated the effect of tributyltin. This inhibitor, which catalyzes a Cl^--OH^- exchange (18, 24), also caused efflux of accumulated transport substrates (Table 4). There were similarities between the effects of Ag^+ and tributyltin in that the Pit and proline uptake systems were affected more than the Pst and glutamine systems (Tables 3 and 4).

We are tempted to speculate that a link in the energy transduction system, affected by both Ag^+ and tributyltin, may be responsible for sustaining an essential basic level of membrane energization serving the maintenance of concentration gradients of transportable metabolites. The known chemistry of Ag^+ and the Cl^--OH^- exchange catalyzed by tributyltin are indicative of the possible involvement of chloride ions. The immobilization of chloride as AgCl would disrupt the cycle of energy transduction by intramembrane protons, as envisaged by Robertson and Boardman (18), thus demolishing the residual energy gradient which is not accessible to uncouplers, which collapse the gradient between the cytoplasm and the bulk proton pool of the medium. It could be expected that Cl^- would be released from the AgCl complex by Br^- and, more readily, by -SH (solubility products for AgCl, AgBr, and Ag_2S are about 10^{-10}, 10^{-13}, and 10^{-50} M, respectively).

Our findings can also be interpreted in the light of a recent report by Plate and Suit (16), who described mutants in the *eup* locus which were able to maintain a normal ΔpH^+, but were unable to couple it to the transport of proline and methyl-β-D-thiogalactopyranoside and, unlike *eup*$^+$ strains, could not transport methyl-β-D-thiogalactopyranoside when an artificial ΔpH was imposed. They postulate a role for the gene product of the *eup* locus in these H^+ symport systems. It is possible that the *eup* product is the target for Ag^+. It should, however, be mentioned that Newman et al. (13) succeeded in demonstrating lactose transport driven by a membrane potential in proteoliposomes reconstituted from *E. coli* lipid and the pure *lacY* gene product. This finding demonstrates that H^+-lactose symport occurs through a single polypeptide. These two reports are at variance, although the possibility is not excluded that the *eup* gene product forms a factor which may be present in the *E. coli* lipid preparation used in the reconstitution. Indeed, proteoliposomes prepared from a less purified preparation of the *lacY* product, but with asolectin as the lipid source, transport lactose at a rate about one-seventh of that observed when *E. coli* phospholipid was used (14).

The total abolition of energy resources must lead to the discharge of all concentration gradients. We envisage that this would be mediated, for each metabolite, by its transport carrier operating as a facilitator. Our finding that phosphate esters (predominantly hexose phosphates and mannitol 1-phosphate [22]) failed to exit

indicates the lack of the relevant facilitator. Indeed, the hexose phosphate uptake system cannot be induced by a substrate present within the cell (6, 28). It should, however, be noted that Ag^+ caused the efflux of P_i at 0°C. At this temperature, neither uptake nor exchange of P_i occurs (22), and this was taken to indicate that the carrier was immobilized at 0°C. It must therefore be assumed that at 0°C Ag^+ may be able to modify the carrier to act as a channel, or else that an independent channel may be formed. In this context, it is interesting to note that Meury et al. (11) have observed efflux of potassium ions from cells upon treatment with *N*-ethylmaleimide and have proposed K^+ channels distinct from the uptake systems.

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