Nucleoside Transport Systems in *Escherichia coli* K12: Specificity and Regulation

AGNETE MUNCH-PETERSEN AND BENTE MYGIND
Institute of Biological Chemistry B, University of Copenhagen, Denmark

ABSTRACT Two nucleoside transport systems have been verified and separated by mating and recombination experiments. The recipient strain was a mutant which is negative for transport of all nucleosides.

The two systems differ in specificity and in regulation. One system transports pyrimidine and adenine nucleosides, but not guanine nucleosides. It is regulated by the *cyt*R gene. The other system transports all nucleosides and is regulated by the *cyt*R as well as by the *deo*R genes.

Enzyme assays performed on whole cells of strains, able or unable to transport nucleosides, indicate that the nucleoside catabolizing enzymes are located inside the permeability barrier of the cell.

Utilization of ribonucleosides and deoxyribonucleosides by cells of *E. coli* K 12 requires transport through the cell membrane, followed by catabolism catalyzed by specific enzymes. Several observations indicate that these enzymes in the cell are located in close association with the cell membrane (Munch-Petersen, '68; Beacham et al., '71; Munch-Petersen and Schwartz, '72) and catabolism of nucleosides has been shown to take place within a few seconds after addition to the cell suspensions (Mygind and Munch-Petersen, '75). Thus the two phases of utilization, transport and catabolism, seem to be tightly coupled in the metabolic apparatus of the cell, and measurement of transport rates are best performed in cells containing mutations in the enzyme which is the first to attack the incoming nucleoside. Alternatively transport may be measured in the presence of an inhibitor of that enzyme.

Using this approach it was shown previously that the overall transport of pyrimidine nucleosides into *E. coli* cells is regulated by the *cyt*R [1] and *deo*R genes (Mygind and Munch-Petersen, '75). These two genes also control the synthesis of the nucleoside catabolizing enzymes (Munch-Petersen et al., '72).

Several authors have reported the presence of two different nucleoside transport systems in the cells of *E. coli* K 12 (Doskočil, '72, Komatsu and Tanaka, '72). Both of these systems are absent in a nucleoside transport negative mutant, isolated in this laboratory by Karin Hammer-Jespersen (unpublished results). Recombinants from this mutant, containing one or the other of the two transport systems, have now been obtained. In the present paper the separate regulation of the two transport systems has been investigated in derivatives in which catabolism of the nucleosides is abolished.

A regulatory pattern was found, according to which the system accommodating all nucleosides is regulated by the *cyt*R gene as well as the *deo*R gene. In contrast the system, which does not transport guanine nucleosides, responds to the control by the *cyt*R gene only.

MATERIALS AND METHODS

Bacterial strains and growth media

The *E. coli* strains employed and their derivation are listed in table 1.

Recombinants, containing the two dif-

[1] *Abbreviations and Symbols*

*cyt*R: regulatory gene for the following enzymes: cytidine deaminase, uridine phosphorylase, deoxyriboaldolase, thymidine phosphorylase, deoxyribomutase and purine nucleoside phosphorylase.

*deo*R: regulatory gene for the *deo* enzymes: deoxyriboaldolase, thymidine phosphorylase, deoxyribomutase and purine nucleoside phosphorylase.

cru and *gru*: symbols for nucleoside transport systems: for specificity and definition see text.

Enzymes

Purine nucleoside phosphorylase or purine nucleoside: orthophosphate (deoxy)ribosyltransferase (EC 2.4.2.1.); thymidine phosphorylase or thymidine: orthophosphate deoxyribosyltransferase (EC 2.4.2.4.); uridine phosphorylase or uridine: orthophosphate ribosyltransferase (EC 2.4.2.3.); cytidine deaminase or (deoxy)cytidine aminohydrolase (EC 3.5.4.5.).

TABLE 1

List of bacterial strains and their derivation

Strain No.	Sex	Relevant genotype	Derivation or Source
SØ 928	Hfr	Δ*deo*	From B. Svenningsen [1]
AM 105	Hfr	Δ*deo*,*cml*A	From SØ 928 [2]
SØ 930	Hfr	Δ*deo*,*deo*R7,*cml*A	From B. Svenningsen [1]
SØ 270	F⁻	*met*B,*cyt*R1	K12 58–561 [3]
SØ 587	F⁻	metB,*arg*A,*lys*A,*cru*,*gru*	K12 58–561 [4]
AM 268	F⁻	*arg*A,*lys*A,*cru*,*gru*,*cyt*R4	From SØ 587[5]
SØ 736	F⁻	*met*B,*cru*	From SØ 587 by mating with Hfr Cavalli
SØ 737	F⁻	*cru*,*cyt*R	From SØ 736 [5]
SØ 738	F⁻	*met*B,*cru*,*deo*R,*cml*A	From SØ 736 by mating with Hfr Hayes (*deo*R7,*cml*A)
AM 145	F⁻	metB,Δ*deo*,*cru*,*cml*A	From SØ 736 [6]
AM 148	F⁻	Δ*deo*,*cru*,*cyt*R4,*cml*A	From SØ 737 [6]
AM 200	F⁻	Δ*deo*,*cru*,*deo*R7,*cml*A	From SØ 736 [7]
AM 234	F⁻	Δ*deo*,*cru*,*cyt*R4,*deo*R7,*cml*A	From SØ 737 [7]
SØ 739	F⁻	*met*B,*gru*,*arg*A,*lys*A	From SØ 587 by mating with Hfr Hayes
SØ 740	F⁻	*gru*,*arg*A,*lys*A,*cyt*R4	From SØ 739 [5]
SØ 741	F⁻	*met*B,*gru*,*arg*A,*lys*A*deo*R7,*cml*A	From SØ 739 by mating with Hfr Hayes (*deo*R7,*cml*A)
AM 146	F⁻	Δ*deo*,*gru*,*met*B,*arg*A,*lys*A,*cml*A	From SØ 739 [6]
AM 149	F⁻	Δ*deo*,*gru*,*arg*A,*lys*A,*cyt*R4,*cml*A	From SØ 740 [6]
AM 238	F⁻	Δ*deo*,*gru*,*met*B,*arg*A,*lys*A,*deo*R7,*cml*A	From SØ 739 [7]
AM 240	F⁻	Δ*deo*,*gru*,*arg*A,*lys*A,*cyt*R4,*deo*R7,*cml*A	From SØ 740 [7]

[1] Svenningsen, '75.
[2] From SØ 928, selecting for spontaneous resistance to chloramphenicol (7.5 μg/ml).
[3] Munch-Petersen et al., '72.
[4] Gift from K. Hammer-Jespersen, this institute.
[5] By transduction with P1v (*met*B⁺,*cyt*R4).
[6] By mating with AM 105, selecting for resistance to chloramphenicol.
[7] By mating with SØ 930, selecting for resistance to chloramphenicol.

ferent transport systems, were isolated in the following way: mating experiments were performed with the transport negative mutant SØ 587 as the recipient and either SØ 599 (Hfr Hayes) or SØ 573 (Hfr Cavalli) as donor. Mating time was 110 minutes and selection was made for growth on cytidine as the sole carbon source.

Use of Hfr Hayes as a donor mainly rendered recombinants which grew on cytidine but not on guanosine, while Hfr Cavalli gave rise to recombinants which grew on guanosine as well as cytidine. The two types of recombinants were purified and tested for growth on and transport of nucleosides and for resistance to the nucleoside analogues fluorodeoxycytidine (Hammer-Jespersen, unpublished) and showdomycin (Komatsu, '71).

Two different recombinants, SØ 736 and SØ 739, which according to growth and resistance properties represented the two different transport systems, were chosen for further analysis. (Details concerning the recombination procedures and the localization on the *E. coli* chromosome of the genes coding for the transport systems will be published elsewhere [Mygind, unpublished]).

The regulatory genes, *cyt*R and *deo*R were introduced into each of the two recombinants. Besides this, a deletion covering the genes coding for thymidine phosphorylase and purine nucleoside phosphorylase was introduced in the different derivatives. These two enzymes are the first to attack thymidine and guanosine, and the introduction of the deletion is especially important in the regulatory strains, since the increased catabolic activity otherwise may interfere with the transport measurements.

Unless otherwise stated the bacteria were grown in phosphate-buffered minimal medium containing $(NH_4)_2\ SO_4$ (15 mM), Na_2HPO_4 (42 mM), NaCl (50 mM), KH_2PO_4 (22 mM), $MgCl_2$ (2 mM), $CaCl_2$ (0.1 mM), and $FeCl_3$ (0.003 mM) and supplemented with the necessary nutritional requirements. Glycerol (0.2%) was used as a carbon source unless otherwise stated.

Genetic manipulations

Mating and recombination experiments

as well as transductions with phage Plv were carried out according to Miller ('72).

The deletion in the deo-operon (Δ*deo*) was introduced by mating with Hfr Hayes donor strains containing this deletion concurrent with a *cmlA* mutation (see list of strains). Selection was made for resistance to chloramphenicol and the recombinants were tested for the absence of thymidine phosphorylase and purine nucleoside phosphorylase by enzyme assay on cell extracts.

Chemicals

Nucleosides, showdomycin, and other fine chemicals were purchased from Sigma Chemical Company (St. Louis, Missouri, U.S.A.). 5-Fluoro-2'-deoxycytidine was a gift from F. Hoffman-La Roche and Co. Ltd. (Basel, Switzerland) through their Danish agents Roche A/S (Copenhagen). Tetrahydrouridine was a generous gift from P. Valentin of this laboratory. It was prepared according to the method of Hanze ('67).

Radioactive isotopes

U-^{14}C-cytidine, U-^{14}C-guanosine and U-^{14}C-thymidine were purchased from the Radiochemical Centre, Ltd (Amersham, England).

Transport assay

Details of the transport assay have been published (Mygind and Munch-Petersen, '75). Cell suspensions were of a density of approximately 2×10^8 cells per ml and glycerol (0.2%) was present as a carbon source.

The radioactive nucleoside was added to a final concentration of $0.6 - 0.7 \times 10^{-6}$M and specific activities approximately 100 μCi per μmole.

Enzyme analysis

Cell extracts were prepared and assayed as described before (Hammer-Jespersen et al., '71).

When assay was performed on whole cells (cytidine deaminase and thymidine phosphorylase) the harvested cells were suspended in minimal medium without a carbon source. From this suspension aliquots were added to the usual assay mixture and the assay was carried out in the same way as with cell extracts.

Units of activity are expressed as nmoles of substrate converted per minute per absorbance unit at 436 nm in the cell suspension used for assay, either directly or after sonic treatment.

Osmotic shock treatment

Cells were grown in minimal medium, containing 0.2% glycerol, and harvested in the exponential phase of growth. The procedure for osmotic shock treatment was that described by Heppel ('68) for cells in the exponential phase. The EDTA-concentration in the sucrose solution was 10^{-4}M. Osmotic shock treatment was carried out with distilled H_2O and with 5×10^{-4}M $MgCl_2$.

Enzyme assays were performed on the shock fluid supernatant, on sonic extracts of the shocked cells and on sonicated extracts of whole cells.

RESULTS

Regulation of the two different nucleoside transport systems

Two different transport systems for nucleosides have been reported in cells of *E. coli* (Doskočil, '72; Komatsu and Tanaka, '72).

One system transports all pyrimidine (deoxy) ribonucleosides, and, to some degree, adenine (deoxy)ribonucleosides, but not guanine (deoxy)ribonucleosides. The other system transports all nucleosides and deoxynucleosides. Strains containing the former system are sensitive to the uridine analogue showdomycin, while strains containing the latter system are resistant. The two systems have mainly been characterized by isolation of mutants resistant to showdomycin (Komatsu and Tanaka, '72).

Stepwise selection of mutants resistant to fluorouracil (2.5 μg/ml). fluorouridine (10 μg/ml), and fluorodeoxycytidine (10 μg/ml) in the order mentioned, has resulted in mutants which totally lack the transport systems for nucleosides (Hammer-Jespersen, unpublished). These mutants do not grow on any nucleoside although they contain a normal complement of the nucleoside catabolizing enzymes. When nucleoside transport is assayed in cell suspensions as described in MATERIALS AND METHODS the transport is negligible. (Insert in fig. 2).

By mating and recombination analysis of such a transport negative mutant, SØ

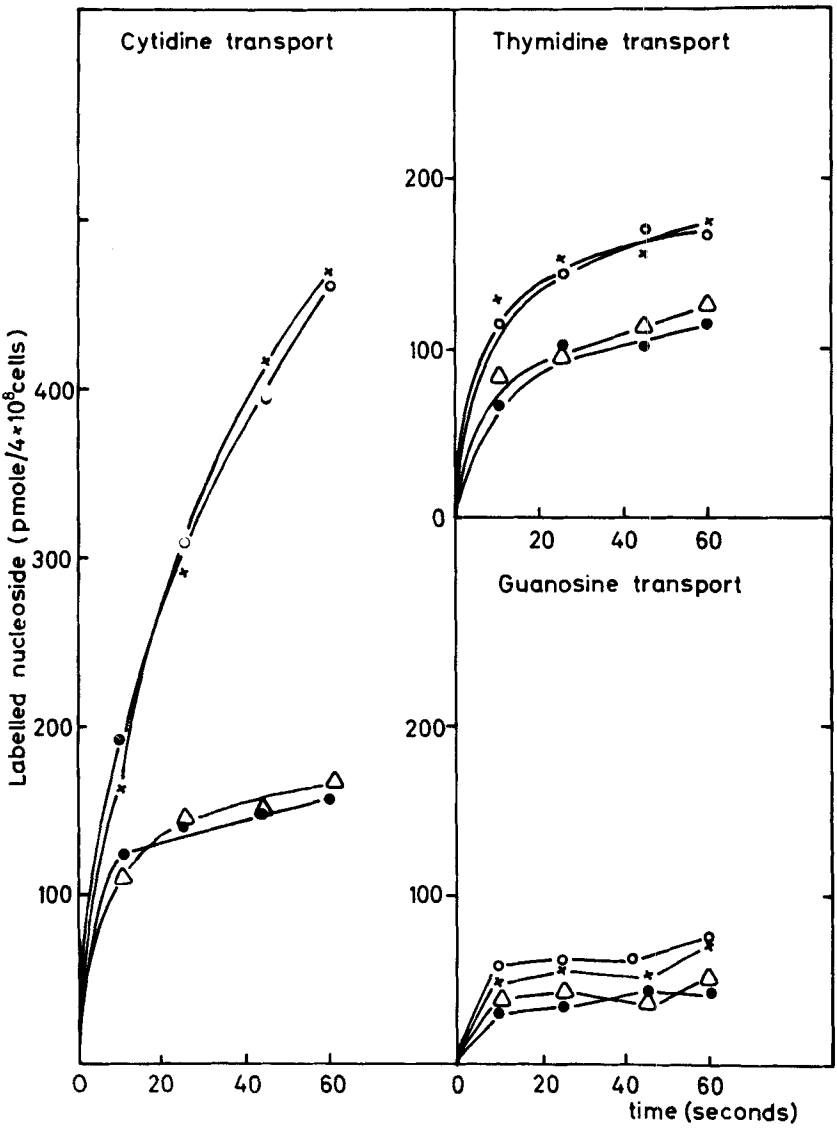

Fig. 1 Transport of nucleosides in different regulatory mutants, containing the *cru*-mediated transport system.

The cells were grown on minimal medium with glycerol as a carbon source and harvested in the logarithmic phase. Cell suspensions of approx. 2 × 10^8 cells per ml were used for transport assay. At zero time the labelled nucleoside (100 μCi per μmole) was added to a final concentration of 6 × 10^{-7}M. At times indicated samples were filtered, washed, dried and counted as described previously (Mygind and Munch-Petersen, '75). Transport is expressed as pmoles labelled nucleoside in 4 × 10^8 cells.

●—●—● AM 146 wt
×—×—× AM 149 *cyt*R
Δ—Δ—Δ AM 238 *deo*R
○—○—○ AM 240 *cyt*R, *deo*R

587, two different types of recombinants have been obtained, corresponding to the two types of transport systems mentioned above. One class contains the transport system which accommodates all nucleosides, while the other class contains the system which allows transport of pyrimidine as well as adenine nucleosides, but not of guanine nucleosides. The gene(s) coding for the former system has tentatively been designated *gru*, while the gene(s) coding for the latter system is termed *cru*.

Cells containing the system coded for by the *gru* gene grow on any nucleoside and they are resistant to showdomycin (0.16 mM). The other type of recombinants, containing the system encoded by the *cru* gene, grow on pyrimidine and adenine nucleosides, but not on guanine nucleosides. These cells are sensitive to showdomycin.

Details of recombination procedures and map position of these genes will be published elsewhere (Mygind, unpublished).

It was found previously (Mygind and Munch-Petersen, '75) that the overall nucleoside transport in *E. coli* cells is controlled by the two regulatory genes, *cyt*R and *deo*R, the same genes which are known to control the synthesis of the nucleoside catabolizing enzymes (Munch-Petersen et al., '72). In order to test the separate control of the two transport systems, regulatory mutations were introduced into the two recombinants SØ 736 (cru^-, gru^+) and SØ 739 (cru^+, gru^-), these two strains representing the two types of transport systems described above.

Interference of catabolism with the transport assay was avoided in the case of thymidine and guanosine by introducing into the recombinants a deletion covering the genes coding for the two enzymes thymidine phosphorylase and purine nucleoside phosphorylase (see METHODS). When cytidine transport was measured, cytidine deaminase was inhibited by the addition of 10^{-4}M tetrahydrouridine, which specifically inhibits this enzyme (Cohen and Wolfenden, '71), without interfering with the cytidine transport (Mygind and Munch-Petersen, '75).

The transport of exogenous cytidine, guanosine and thymidine was measured in the two series of strains, i.e., a cru^+, gru^- series and a cru^-, gru^+ series, each with the following regulatory background: wild-type, *cyt*R, *deo*R, and *cyt*R, *deo*R (see list of strains). The results are shown in figures 1 and 2.

In the cru^+ gru^--recombinants the transport of guanosine is negligible in the wild type derivative as well as in the regulatory strains (fig. 1). In contrast the cells transport and concentrate cytidine and, although less efficiently, thymidine.

Comparison between the four transport curves shows that a *deo*R mutation has no effect on the rate of transport, while the *cyt*R mutation gives rise to a significant increase in rate. The maximal nucleoside concentration in the cells as compared to the medium is found in the *cyt*R derivative. Calculated according to Vorisek and Kepes ('72), the concentration ratios are 1400 fold and 600 fold for cytidine and thymidine, respectively, at an exogenous nucleoside concentration of 6×10^{-7}M.

The cru^- gru^+ recombinants are able to transport guanosine as well as cytidine and thymidine (fig. 2). This system is regulated by the *cyt*R gene as shown by the raised rates of transport for the three nucleosides in the *cyt*R derivative. The maximal ability to concentrate nucleosides is, however, found in the double regulatory derivative (AM 234, *cyt*R, *deo*R), indicating that the *gru*-mediated transport system is controlled by both regulatory genes. In this strain guanosine, added in the medium at a concentration of 6×10^{-7}M is concentrated approximately 900 fold in the cells. The maximal concentrating ability for cytidine and thymidine (also found in the double mutant) is similar to that found in cells containing the *cru*-mediated, *cyt*-R-controlled transport system.

Catabolism of nucleosides by whole cells

The inability of the transport negative mutant, SØ 587, to grow on any nucleoside indicates that a transport process must occur prior to the complete degradation of the nucleoside. This does not, however, exclude the possibility that the initial enzymic attack on the nucleoside may occur outside the cells' permeability barrier. Several reports have indicated that the nucleoside catabolizing enzymes are released to a certain degree when the cells are given an osmotic shock. They are, how-

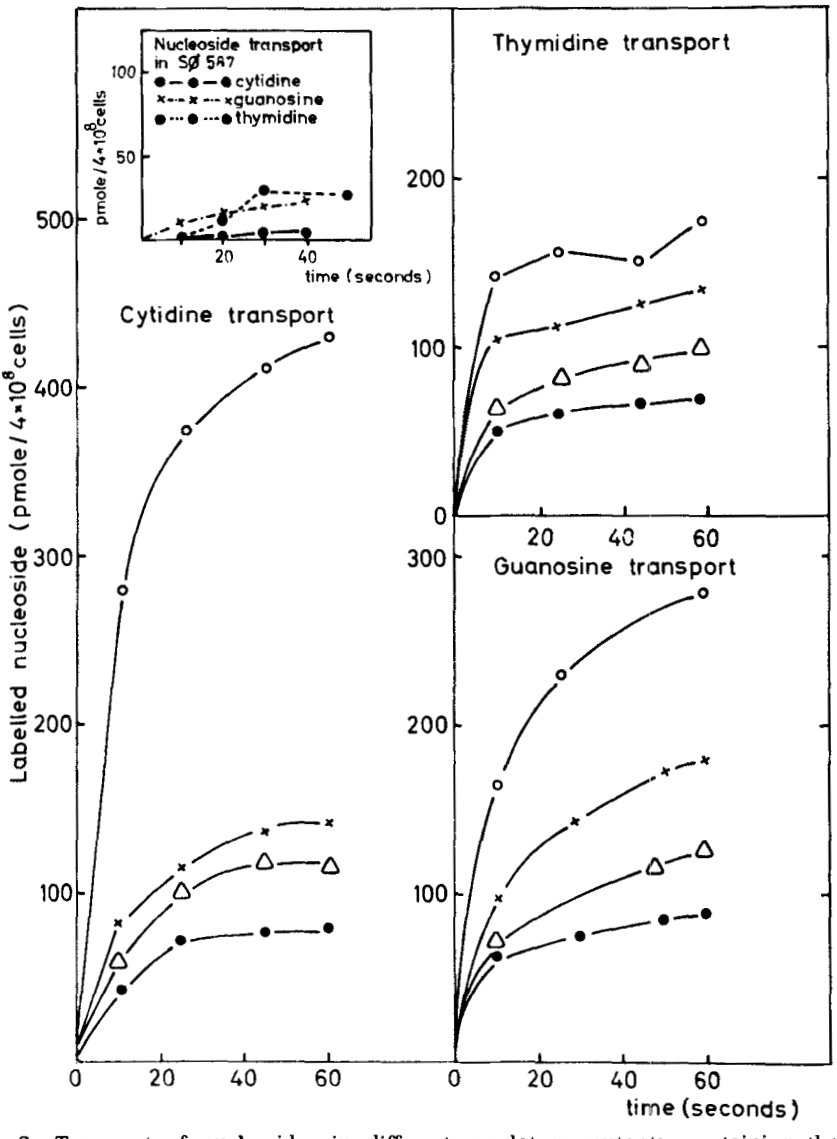

Fig. 2 Transport of nucleosides in different regulatory mutants, containing the *gru*-mediated transport system.

Cell growth and transport assay were carried out as described in legend to figure 1.

●—●—● AM 145 wild type
×—×—× AM 148 *cyt*R
Δ—Δ—Δ AM 200 *deo*R
○—○—○ AM 234 *cyt*R, *deo*R

Insert in figure 2. Transport of cytidine, guanosine and thymidine, as measured in the transport negative strain SØ 587. Cell growth and enzyme assays were performed as described in the legend to figure 1.

ever, not released by lysozyme treatment (Beacham et al., '71), as are the true periplasmic enzymes (Heppel, '71).

Table 2 shows the amounts of enzymes, which are released after osmotic shock treatment of a strain which harbours a *cyt*R mutation and therefore contains high levels of nucleoside catabolizing enzymes.

TABLE 2

The release of nucleoside catabolizing enzymes by osmotic shock. The effect of the presence of 0.5 mM $MgCl_2$ in the shock fluid.

	Enzyme Activities (units/A_{436} unit)							
	Uridine phosphorylase		Cytidine deaminase		Thymidine phosphorylase		Purine nucleoside phosphorylase	
	H_2O	0.5 mM $MgCl_2$	H_2O	0.5 mM $MgCl_2$	H_2O	0.5 mM $MgCl_2$	H_2O	0.5 mM $MgCl_2$
Supernatant of shocked cells	21 (24)	13 (14)	137 (81)	24 (17)	16 (76)	3 (16)	5 (31)	7 (39)
Resuspended shocked cells (sonicated)	67 (76)	80 (86)	32 (19)	117 (83)	5 (24)	16 (84)	11 (69)	11 (61)
Total activity in shock fluid + shocked cells	88	93	169	141	21	19	16	18
Sonicated cell extracts	108		171		18		15	

Strain SØ 270, *met*B,*cyt*R, was grown in minimal medium with glycerol as a carbon source and harvested in the exponential phase of growth. The osmotic shock was performed as described by Heppel ('68), see MATERIALS AND METHODS. Enzyme assays were performed as described (Hammer-Jespersen et al., '71). Enzyme activities are given as units per absorbance unit at 436 nm in the original culture. Figures in parenthesis are percent activity of total activity recovered in the supernatant of shocked cells and in the extracts of shocked cells. Similar results were obtained with a wild-type strain, uninduced or induced with cytidine 1 mg/ml (Hammer-Jespersen et al., '71) in the culture medium (Data not shown).

TABLE 3

Enzyme activities, measured in whole cells and sonic extracts

Strain No.	relevant genotype	cytidine deaminase units/A_{436} unit		thymidine phosphorylase units/A_{436} unit	
		whole cells	sonic extracts	whole cells	sonic extracts
AM 268	*cru,gru,cyt*R	5	320	5	212
SØ 737	*cru,cyt*R	38	297	12	290
SØ 738	*cru,deo*R	8.5	28	28	432
AM 234	*cru,cyt*R,*deo*R,Δ*deo*	160	370	—	—
SØ 740	*gru,cyt*R	30	367	14	230
SØ 741	*gru,deo*R	7.2	37	22	486
AM 240	*gru,cyt*R, *deo*R,Δ*deo*	43	410	—	—

Cells were grown overnight on limited carbon source (0.04 % glycerol). They were harvested, washed with minimal medium and suspended in the same medium at a cell density of 1.2×10^9 cells per ml. Analyses were performed using either whole cell suspensions or sonic extracts of the cells as described in MATERIALS AND METHODS.

The four enzymes, cytidine deaminase, thymidine phosphorylate, uridine phosphorylase and purine nucleoside phosphorylase were investigated. Each of these enzymes is the first to attack specifically when the appropriate nucleoside is offered to the cell, the nucleosides being cytidine, thymidine, uridine and guanosine respectively. When the shock fluid is distilled water, the enzymes fall in two groups. Cytidine deaminase and thymidine phosphorylase are released 80%, while of uridine phosphorylase and purine nucleoside phosphorylase only approximately 15% are set free. If 0.5 mM $MgCl_2$ is added to the shock fluid, none of the enzymes are released in appreciable amounts.

The relative ease with which cytidine deaminase and thymidine phosphorylase are released by distilled water poses the question whether these two enzymes are, in fact, located outside the permeability barrier of the cell so that in the transport negative strain deamination or phosphorolysis might still occur, but in such a way that the products are spilled into the medium instead of being utilized by the cells.

To test this possibility the deamination of cytidine and the phosphorolysis of thymidine were determined in cells with or

without a functioning transport system. The results are given in table 3. Here the enzymic activities of whole cells are compared with the corresponding activities in sonic extracts of the same cells. These analyses were performed on the regulatory derivatives used for transport assays. In the transport negative *cyt*R strain deamination of cytidine and phosphorolysis of thymidine by whole cells are both negligible, in spite of high cellular levels of the two enzymes. Introduction of one or the other of the two transport systems enables the cell to carry out the enzymic attack on the two nucleosides. The results for cytidine deaminase in the double mutants furthermore confirm the regulatory pattern found in the transport assays (fig. 1). The double regulatory mutant containing the *gru*-linked transport system deaminates cytidine at a much faster rate than does the *cyt*R strain, while in the strain containing the *cru*-linked system, the double mutant behaves like the *cyt*R mutant. In all strains the enzyme activities of the whole cells are much lower than those found in sonic cell extracts, indicating that in general the rate of transport of the nucleosides into the cells is the rate-limiting factor for enzymic attack on incoming nucleosides.

DISCUSSION

The tight coupling between transport and catabolism of nucleosides in bacterial cells tends to complicate investigations of the transport systems proper. In fact, it has been suggested that in membrane vesicles of *S. typhimurium* (Hochstadt, '74) and of transformed 3T3 cells (Quinlan and Hochstadt, '76) the nucleoside phosphorylases may act as mediators for the transport of uridine and inosine respectively in carrying out a group translocation reaction.

The results in the present paper seem to exclude that such a group translocation is required for transport of nucleosides in *E. coli*. The evidence is the following:

1. Strain SØ 587, which has a full complement of nucleoside catabolizing enzymes does not grow on or transport any nucleoside.

2. Recombinants of this strain may be constructed, which are capable of transport of and growth on nucleosides. The levels of nucleoside catabolizing enzymes are unchanged in these recombinants.

3. Strains with deletions or point mutations (Mygind and Munch-Petersen, '75) in the nucleoside catabolizing enzymes are able to transport and concentrate nucleosides added to the medium.

Furthermore it is shown, in the case of cytidine and thymidine, that the catabolic enzymes are not able to attack these nucleosides unless a transport system is functioning in the cells.

Two different transport systems for nucleosides have previously been characterized in *E. coli* (Komatsu and Tanaka, '72) and they have been shown to function in both whole cells and in preparations of vesicles (Komatsu and Tanaka, '72).

In the present work the two transport systems have been separated by mating and recombination techniques, using a transport negative mutant as recipient. Besides the previously known difference in specificity (Komatsu and Tanaka, '72) the results in figures 1 and 2 show that the two systems differ in regulatory properties in such a way that the *cyt*R gene controls the synthesis of one or more components of both transport systems, while only the system encoded by the *gru* gene(s) responds to control by the *deo*R gene. This agrees with previous results (Mygind and Munch-Petersen, '75) that the overall transport of pyrimidines nucleosides in *E. coli* cells is controlled by both regulatory genes. The *deo*R control exerted on the *gru*-mediated transport further suggests that this system is identical to the thymidine inducible nucleoside transport system, described by Doskočil ('74).

None of the two transport systems has as yet been resolved into single components, and it is not clear which components are the targets of control. Neither is it known whether the two systems have one or more common factor(s).

The fact that transport and catabolism of nucleosides are controlled by the same regulatory genes emphasizes the tight coupling between the two stages of utilization. The partial, but quite specific (Beacham et al., '71) release of the catabolizing enzymes by osmotic shock treatment may indicate that these enzymes in some way are associated with the inside surface of the cytoplasmic membrane. One might visualize a spatial as well as a regulatory relationship between transport apparatus and catabolizing enzymes in such a way

that the immediate catabolism of nucleosides takes place in close connection with the cell membrane, in which the transport systems reside.

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LITERATURE CITED

Beacham, I. R., E. Yagil, K. Beacham and R. H. Pritchard 1971 On the localization of enzymes of deoxynucleoside catabolism in *Escherichia coli*. FEBS Letters, *16*: 77–80.

Cohen, P. M., and R. Wolfenden 1971 Cytidine deaminase from *Escherichia coli*. J. Biol. Chem., *246*: 7561–7565.

Doskočil, J. 1972 The components of the nucleoside-transporting system in *Escherichia coli*. Biochim. Biophys. Acta, *282*: 393–400.

——— 1974 Inducible nucleoside permease in *Escherichia coli*. Biochem. Biophys. Res. Comm., *56*: 997–1003.

Hammer-Jespersen, K., A. Munch-Petersen, P Nygaard and M. Schwartz 1971 Induction of enzymes involved in the catabolism of deoxyribonucleosides and ribonucleosides in *Escherichia coli* K 12. Eur. J. Biochem., *19*: 533–538.

Hanze, A. R. 1967 Nucleic acids IV. The catalytic reduction of pyrimidine nucleosides. J. Am. Chem. Soc., *89*: 6720–6725.

Heppel, L. 1968 Methods in Enz. L. Grossman and K. Moldave, eds. Academic Press, New York and London, Vol. XIIB, pp. 841–846.

——— 1971 Structure and Function of Biological Membranes. L. Rothfield, ed. Academic Press, New York and London, pp. 223–247.

Hochstadt, J. 1974 The role of the membrane in the utilization of nucleic acid precursors. CRC Crit. Rev. Biochem., *2*: 259–310.

Komatsu, Y. 1971 Mechanism of action of showdomycin. J. Antibiotics, XXIV: 876–883.

Komatsu, Y. and K. Tanaka 1972 A showdomycin resistant mutant of *Escherichia coli* K12 with altered nucleoside transport character. Biochim. Biophys. Acta, *288*: 390–403.

Miller, J. 1972 Experiments in molecular genetics. Cold Spring Harbor Laboratory.

Munch-Petersen, A. 1968 On the catabolism of deoxyribonucleosides in cells and cell extracts of *Escherichia coli*. Eur. J. Biochem., *6*: 432–442.

Munch-Petersen, A., P. Nygaard, K. Hammer-Jespersen and N. Fiil 1972 Mutants constitutive for nucleoside catabolizing enzymes. Eur. J. Biochem., *27*: 208–215.

Munch-Petersen, A., and M. Schwartz 1972 Inhibition of the catabolism of deoxyribonucleosides in *Escherichia coli* after infection by T_4-phage. Eur. J. Biochem., *27*: 443–447.

Mygind, B., and A. Munch-Petersen 1975 Transport of pyrimidine nucleosides in cells of *Escherichia coli* K 12. Eur. J. Biochem., *59*: 365–372.

Quinlan, D. C., and J. Hochstadt 1976 Group translocation of the ribose moiety of inosine by vesicles of plasma membrane from 3T3 cells, transformed by simian virus 40. J. Biol. Chem., *251*: 344–354.

Roy-Burman, S., Y. H. Huang and D. W. Visser 1971 Inhibition of amino acid and sugar transport by showdomycin. Biochem. Biophys. Res. Comm., *42*: 445–453.

Svenningsen, B. 1975 Regulated in vitro synthesis of the enzymes of the *deo* operon of *Escherichia coli*. Molec. gen. Genet., *137*: 289–304.

Vorrisek, J., and A. Kepes 1972 Galactose transport in *Escherichia coli* and the galactose binding protein. Eur. J. Biochem., *28*: 364–372.