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TRANSPORT STUDIES OF SHOWDOMYCIN, NUCLEOSIDES AND SUGARS IN *ESCHERICHIA COLI* B AND IN SHOWDOMYCIN-RESISTANT MUTANTS

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SUMMARY

Competition between the nucleoside antibiotic, showdomycin, and certain naturally occurring nucleosides for a common transport system can explain the protective action of the nucleosides against inhibition by showdomycin of growth, amino acid transport and sugar transport in *Escherichia coli* B. Uridine interferes with the transport of showdomycin into the cell, thereby preventing the inhibitory effects of the antibiotic. With the exception of adenosine, nucleosides which protect against inhibition by showdomycin are mutually competitive for their transport. Guanosine, which does not protect against the inhibitory effect of showdomycin on glucose transport, does not compete for the transport of the protective nucleosides, uridine, cytidine or adenosine. Adenosine inhibits transport of uridine, cytidine and guanosine, but appears to have separate transport requirements. A mutant of *E. coli* B resistant to 80 μM showdomycin exhibits normal growth and glucose transport properties. The transport of uridine, cytidine and showdomycin is markedly reduced in the mutant, whereas guanosine and adenosine transport is similar to that in wild-type cells. Exogenous showdomycin, which inhibits transport of glucose, guanosine and protective nucleosides in *E. coli* B, has no effect on these processes in mutant cells. A defect in the capacity of the mutant to transport showdomycin explains the resistance of the mutant to the inhibitory effects of showdomycin. The inhibitory effects of *N*-ethylmaleimide, in contrast to showdomycin, are similar in mutant and *E. coli* B cells.

INTRODUCTION

The nucleoside antibiotic, showdomycin, inhibits growth, protein and nucleic acid synthesis, and the transport of sugars and amino acids in *Escherichia coli*[1-3]. These inhibitory effects are reversed completely by thiol compounds and by most of the common nucleosides. The protective action of thiol compounds against showdomycin inhibition is readily explained since showdomycin exerts its inhibitory effects by alkylation of susceptible sulfhydryl groups[1-4]. A basis for the protective action of nucleosides on showdomycin inhibition of sugar and amino acid transport is less

obvious. It seems probable that these protective effects are related directly to the nucleoside-like structure of the antibiotic since naturally occurring nucleosides do not protect against the action of the alkylating agent, *N*-ethylmaleimide, which is structurally and functionally similar to the aglycon moiety of showdomycin[3,5]. Thus, a logical possibility is that nucleosides may interact at specific sites on the cell surface, thereby preventing transport of the structurally related antibiotic and its subsequent reaction with susceptible sulfhydryl groups. This hypothesis served as the basis for this investigation of nucleoside transport in *E. coli* B in relationship to the effects of showdomycin. The results show that showdomycin inhibits nucleoside transport and uridine inhibits showdomycin transport. Supportive evidence for the conclusions was obtained from studies of sugar and nucleoside transport in showdomycin-resistant *E. coli* B cells.

MATERIALS AND METHODS

The ^{14}C-labeled compounds were obtained from New England Nuclear. Showdomycin (30 mg) was ^{3}H-labeled (200 mCi) by New England Nuclear. The product was separated from impurities as described below.

Purification of ^{3}H-labeled showdomycin

After removal of water and ethanol, the dried product was dissolved in a minimum amount of water and chromatographed on Whatman No. 3 MM filter paper in Solvent A consisting of 0.5 M ammonium acetate (pH 6.5) and ethanol (2:5, by vol.). The ultraviolet-absorbing area of the chromatogram corresponding to showdomycin was cut out, eluted with water and lyophilized. The lyophilized product was chromatographed on Whatman No. 3 MM paper using the upper phase of Solvent B composed of *n*-butanol–acetic acid–water (5:1:4, by vol.). The product was eluted from the region of paper corresponding to showdomycin and lyophilized. The residue was further purified by chromatography on Whatman No. 3 MM paper using Solvent A. The R_F values of showdomycin in Solvent A and in the upper phase of B were 0.77 and 0.46, respectively. The final product (3 % yield) gave a single radioactive peak corresponding to authentic showdomycin in both solvents.

Isolation of showdomycin-resistant mutants

An *E. coli* B (Hill) mutant resistant to 80 μM showdomycin was developed as described by Beljanski *et al.*[6] by sequential passage of cells in minimal medium[7] containing 20, 40 and 80 μM showdomycin. Mutants resistant to 40 μM and 80 μM showdomycin are referred to in the text as mutant A and mutant C, respectively. Mutant C grows as well in 80 μM showdomycin as the wild type in its absence, and retains its sensitivity to phages T_1, T_2 and T_3. The mutants and the parent cells used in all experiments were grown in minimal medium[7] in the absence of showdomycin and were harvested as described previously[3].

Assay for uptake

Unless otherwise noted, the standard reaction mixture (1 ml) contained 0.2 mM ^{14}C-labeled sugar or 0.25 mM ^{14}C-labeled nucleoside and a cell suspension of *E. coli* B or the showdomycin-resistant mutants (equivalent to 0.4–0.5 mg dry weight) in

Medium A*. The specific activities of the sugars and nucleosides used were as follows: [U-^{14}C]glucose (0.05 μCi/μmole); [1-^{14}C]2-deoxyglucose (0.5 μCi/μmole); [U-^{14}C]-uridine (0.5 μCi/μmole); [U-^{14}C]cytidine (0.8 μCi/μmole); [8-^{14}C]adenosine (0.8 μCi/μmole); and [8-^{14}C]guanosine (0.8 μCi/μmole). After incubation at 37 °C in the presence of radioactive sugar or nucleoside for 10 min, the reaction mixtures were chilled, diluted immediately with 5 ml of ice-cold Medium A and filtered through a Millipore filter (0.45 μm pore size). The filters were washed and counted as described previously[3]. A zero time control[3] (usually 30–100 cpm) was included for each set of experiments and subtracted to obtain the data presented in the tables. Each experiment was done in duplicate and repeated 3–5 times. In some experiments as indicated, the measurements of radioactivity were carried out in a Beckman liquid scintillation spectrometer, Model No. LS 245, using a scintillation fluid containing 5 g of PPO and 300 mg of POPOP in 1 l of toluene. The data represent the total uptake of radioactive compound into the cell, including incorporation into acid-soluble compounds and nucleic acids.

RESULTS

The concentration of uridine required to reverse completely the inhibitory effect of showdomycin on glucose transport is approximately proportional to the concentration of the antibiotic over an 8-fold concentration range (Table I). For example, inhibition of glucose transport by 0.02 mM showdomycin is prevented completely by 0.5 mM uridine, whereas in the presence of 0.16 mM showdomycin, 5.0 mM uridine is required for complete protection.

TABLE I

EFFECT OF URIDINE ON GLUCOSE TRANSPORT IN *E. coli* B INHIBITED BY SHOWDOMYCIN

Uptake was measured after 10 min incubation at 37 °C under standard assay conditions, except that prior to the addition of [^{14}C]glucose, the reaction mixtures were preincubated for 10 min at 37 °C with uridine at the concentrations indicated, then showdomycin as indicated was added and the preincubation was continued for an additional 10 min. Glucose uptake in the absence of showdomycin and uridine was taken as 100%. —, not determined.

Uridine added (mM)	*Glucose uptake* (%)			
	Without showdomycin	*0.02 mM showdomycin*	*0.08 mM showdomycin*	*0.16 mM showdomycin*
None	100 (126) *	10	7	5
0.5	101	100	50	4
1.0	100	—	90	49
5.0	100	—	100	96

* Values in the parentheses represent nmoles of glucose incorporated.

The effect of various concentrations of showdomycin on the transport of nucleosides in the presence and absence of the energy source, mannose, is given in Table II. Nucleoside uptake is linear with time at each condition. Mannose increases the uptake of uridine, adenosine and guanosine 4–6-fold, whereas cytidine uptake is stimulated

* Minimal medium described by Davis and Mingioli[7] without glucose.

TABLE II

EFFECT OF SHOWDOMYCIN ON THE UPTAKE OF NUCLEOSIDES IN THE PRESENCE AND ABSENCE OF MANNOSE

Uptake was measured after 10 min incubation at 37 °C under standard assay conditions, except that prior to the addition of [^{14}C]nucleoside, the reaction mixtures were preincubated for 10 min at 37 °C with or without mannose, then showdomycin as indicated was added and the preincubation was continued for an additional 5 min. Nucleoside uptake in the absence of mannose and showdomycin was taken as 100%. —, not determined.

[^{14}C]*Nucleoside*	*Mannose added*	*Uptake* (%)					
		No showdomycin	*0.02 mM showdomycin*	*0.04 mM showdomycin*	*0.08 mM showdomycin*	*0.16 mM showdomycin*	*0.40 mM showdomycin*
Uridine	None	100	68	55	51	32	15
	1.0 mM	461	28	22	22	19	—
Cytidine	None	100	38	—	18	11	9
	1.0 mM	112	8	—	6	—	—
Guanosine	None	100	50	—	45	47	42
	1.0 mM	650	44	—	40	45	41
Adenosine	None	100	78	67	62	36	33
	1.0 mM	611	67	50	44	24	23

about 15%. Although not shown in Table II, the active nature of nucleoside transport is also demonstrated by a similar increase of uptake by the addition of glucose and the failure of 2-deoxyglucose to stimulate uptake. This is consistent with the findings of Peterson and Koch[8] and Peterson *et al.*[9], who showed that nucleoside transport in *E. coli* is inhibited by compounds such as sodium azide and 2,4-dinitrophenol. Nucleosides also stimulate transport, probably because they may serve as efficient energy sources for transport of those nucleosides which do not compete for a common transport site. For example, cytidine stimulates transport of guanosine and adenosine about 2–4-fold in the absence of mannose (Table III). Pseudouridine does not stimulate transport of any nucleoside tested, presumably because it is not a substrate for uridine phosphorylase (EC 2.4.2.3.). Showdomycin inhibits the transport of uridine, cytidine and adenosine to a maximum of about 80–90%, but a much higher concentration of showdomycin (at least 10-fold) is required than that necessary for an equivalent inhibition of glucose transport (Tables I and II). Transport of guanosine is less sensitive to inhibition by showdomycin than the other nucleosides tested, and maximum inhibition at high concentrations of the antibiotic is limited to about 60%. Preincubation with mannose increases the inhibitory effect of showdomycin on nucleoside uptake. The antibiotic not only abolishes the stimulatory effect of mannose, but also inhibits nucleoside uptake to a level slightly below that observed in the absence of mannose. The inhibitory effects of showdomycin on nucleoside uptake are also greater when the antibiotic is preincubated with the cells prior to the addition of nucleoside. For example, preincubation increases the inhibitory effect of showdomycin (0.16 mM) on uridine uptake from 56 % to 68 % (Tables II and V), and adenosine uptake from zero to about 60 % (Table V).

The effect of various nucleosides on the uptake of uridine, cytidine, adenosine and guanosine is shown in Table III. Since transport of nucleosides is energy-depen-

TABLE III

THE EFFECT OF VARIOUS NUCLEOSIDES ON THE UPTAKE OF GUANOSINE, URIDINE, CYTIDINE AND ADENOSINE IN *E. coli* B CELLS

Nucleoside uptake was measured after 10 min incubation at 37 °C under standard assay conditions, except that prior to the addition of ^{14}C-labeled nucleoside, the reaction mixtures were preincubated for 10 min at 37 °C with 1.0 mM mannose followed by 10 min preincubation with 1.0 mM nucleoside as indicated. —, not determined.

Addition	*Cytidine uptake (nmoles)*	*Uridine uptake (nmoles)*	*Adenosine uptake (nmoles)*	*Guanosine uptake (nmoles)*
None	37.5 (100)	6.6 (100)	6.3 (100)	4.0 (100) *
Cytidine	—	1.4 (22)	13.6 (216)	6.3 (158)
Uridine	7.1 (19)	—	11.4 (181)	5.2 (129)
Adenosine	10.6 (28)	6.2 (93) ***	—	2.1 (53)
Guanosine	36 (96)	6.7 (101)	5.3 (84)	—
Pseudouridine	40.6 (108)	6.4 (97)	6.8 (109)	4.1 (102)
None (−mannose) **	24.4 (65)	2.1 (28)	1.5 (24)	0.7 (18)
Cytidine (−mannose) **	—	0.5 (8)	3.1 (48)	2.9 (72)

* Values in the parentheses represent per cent uptake. Nucleoside uptake values in the absence of any other added nucleoside were taken as 100%.

** The reaction mixture did not contain mannose, otherwise the conditions were same as above.

*** 0.9 nmole of uridine was incorporated in the absence of mannose.

TABLE IV

EFFECT OF SHOWDOMYCIN ON THE TRANSPORT OF SUGARS IN *E. coli* B CELLS AND IN SHOWDOMYCIN-RESISTANT MUTANTS A* AND C**

Uptake was measured after 10 min incubation at 37 °C under standard assay conditions, except that prior to the addition of [^{14}C]glucose or [^{14}C]-2-deoxyglucose the reaction mixtures were preincubated for 5 min at 37 °C with *N*-ethylmaleimide or showdomycin at indicated concentrations. Sugar uptake obtained in the absence of showdomycin and *N*-ethylmaleimide was taken as 100%.

^{14}C-labeled substrate	Addition (μM)	Sugar uptake (%)		
		E. coli B	*Mutant A*	*Mutant C*
Glucose	None	100 (112)***	100 (102)	100 (105)
	Showdomycin (20)	15	100	90
	Showdomycin (40)	5	—	100
	Showdomycin (80)	7	80	95
	Showdomycin (160)	4	64	85
	N-Ethylmaleimide (80)	12	16	—
2-Deoxyglucose	None	100 (8.6)	100 (8.0)	100 (7.6)
	Showdomycin (80)	29	97	—
	Showdomycin (160)	30	74	—

* Resistant to 40 μM showdomycin.
** Resistant to 80 μM showdomycin.
*** Values in the parentheses indicate the nmoles of sugar incorporated.

TABLE V

EFFECT OF SHOWDOMYCIN AND *N*-ETHYLMALEIMIDE ON THE UPTAKE OF NUCLEOSIDES IN *E. coli* B AND SHOWDOMYCIN-RESISTANT MUTANT C

Uptake was measured after 10 min incubation at 37 °C under standard assay conditions. *N*-Ethylmaleimide was added at the same time as [^{14}C]nucleosides. Showdomycin was added at the same time as [^{14}C]cytidine and -uridine, whereas the reaction mixtures were preincubated with showdomycin for 5 min at 37 °C prior to the addition of [^{14}C]adenosine and [^{14}C]guanosine. Nucleoside uptake in *E. coli* B cells in the absence of showdomycin and *N*-ethylmaleimide was taken as 100%. —, not determined.

[^{14}C]Nucleoside	Nucleoside uptake (%)					
	E. coli B			*Mutant C*		
	No addition	*With showdomycin (0.16 mM)*	*With N-ethyl-maleimide (0.16 mM)*	*No addition*	*With showdomycin (0.16 mM)*	*With N-ethyl-maleimide (0.16 mM)*
Uridine	100 (3.1)*	44	44	46 (1.4)	50	13
Cytidine	100 (26)	29	—	25 (6.5)	26	—
Guanosine	100 (0.96)	45**	43	90 (0.94)	94	48
Adenosine	100 (2.0)	40**	41	95 (1.9)	90	38

* Values in the parentheses represent nmoles of nucleoside incorporated.
** Adenosine and guanosine uptake is 100% and 57%, respectively, when showdomycin is added without preincubation.

dent and nucleosides themselves can serve as an energy source, these studies were carried out in the presence of 1.0 mM mannose to minimize the stimulatory effects of the unlabeled nucleosides. Qualitatively, the results are the same in the presence or absence of mannose with one exception indicated below. Cytidine and uridine are mutually inhibitory, whereas guanosine and pseudouridine have no effect on their transport. Adenosine inhibits cytidine transport in the presence or absence of mannose, but adenosine inhibits uridine transport only in the absence of the energy source. The uptake of cytidine is about five times greater than that of uridine or adenosine and about nine times that of guanosine uptake.

The effects of other nucleosides on adenosine and guanosine uptake are markedly different from those observed on pyrimidine nucleoside uptake (Table III). Adenosine transport is unaffected by pseudouridine, slightly inhibited by guanosine, and stimulated by the pyrimidine nucleosides. Guanosine uptake is inhibited adenosine and stimulated by the pyrimidine nucleosides.

Mutants of *E. coli* B which are resistant to 40 μM (mutant A) and 80 μM (mutant C) showdomycin were developed. These mutants were compared with *E. coli* B for their ability to transport glucose or 2-deoxyglucose in the presence of various concentrations of showdomycin (Table IV). The mutant cells grow normally and their capacity to transport glucose or deoxyglucose is only slightly reduced as compared to *E. coli* B. The resistance of the mutants to concentrations of showdomycin which completely inhibit growth of *E. coli* B correlates with the unimpaired capacity of the mutants to transport sugars in the presence of showdomycin. For example, glucose transport is not affected by 80 μM showdomycin in mutant C, whereas 20 μM showdomycin inhibits glucose transport 85 % in *E. coli* B.

The effects of showdomycin and *N*-ethylmaleimide on the uptake of nucleosides in *E. coli* B and showdomycin-resistant mutant C are compared in Table V. The capacities for guanosine and adenosine transport by mutant C are similar to those of *E. coli* B, whereas uridine and cytidine are transported much less efficiently by mutant cells than by *E. coli* B. Marked differences between the mutant and *E. coli* B are also apparent in the effects of showdomycin and *N*-ethylmaleimide on nucleoside transport. *N*-ethylmaleimide inhibits nucleoside transport to a similar extent in both mutant and normal cells, whereas showdomycin has no effect on nucleoside transport in mutant cells, but in *E. coli* B it has an inhibitory effect comparable to *N*-ethylmaleimide.

The uptake of [^{3}H]showdomycin is significantly lower in resistant cells than in *E. coli* B (Table VI). In both cases the apparent uptake of showdomycin at zero time and 0 °C is high, about one-third that obtained with *E. coli* B after a 10-min incubation period. These high values for the zero time control may be due to chemical interaction of [^{3}H]showdomycin with exposed sulfhydryl groups of the cell wall or membrane. Uridine, but not guanosine, prevents the uptake of showdomycin by *E. coli* B during the incubation period. However, in mutant cells neither guanosine nor uridine has an effect on showdomycin uptake.

Since guanosine uptake is low in the absence of mannose as compared to transport of uridine, cytidine and adenosine (Table III), it was of importance to eliminate this low uptake as a possible explanation for the inability of guanosine to reverse the showdomycin effect. Therefore, the relative ability of guanosine and uridine to reverse the inhibitory effect of showdomycin on glucose transport was determined in the

presence of mannose which stimulates guanosine uptake 6-fold (Table III). The results establish that guanosine is ineffective as a reversing agent in the presence or absence of mannose, whereas uridine is effective as a protective agent under both conditions.

TABLE VI

SHOWDOMYCIN UPTAKE BY *E. coli* B CELLS AND SHOWDOMYCIN-RESISTANT MUTANT C*

The reaction mixture (1 ml) contained 0.2 mM [^{3}H]showdomycin ($2.6 \cdot 10^5$ cpm/μmole) and cell suspension (equivalent to 0.4 mg dry weight) of *E. coli* B or showdomycin-resistant mutant C in medium A. Prior to the addition of [^{3}H]showdomycin the reaction mixtures were preincubated for 10 min at 37 °C with or without uridine or guanosine as indicated. Uptake was measured after 10 min incubation at 37 °C under standard assay conditions described in the text. —, not determined.

Addition (mM)	*Incubation period (min)*	*Showdomycin uptake (cpm)*	
		E coli B	*Mutant C*
None	0**	270	250
None	10	655	380
Uridine (0.5 mM)	10	389	—
Uridine (5.0 mM)	10	234	390
Guanosine (1.0 mM)	10	560	391

* Mutant resistant to 80 μM showdomycin.

** [^{3}H]showdomycin was added to the ice-cold reaction mixture and immediately diluted, filtered and washed as indicated in the text.

DISCUSSION

The data provide conclusive evidence that uridine interferes with the transport of showdomycin into *E. coli* B cells, thereby preventing interaction of the antibiotic with sulfhydryl groups of proteins necessary for transport processes. The protective nucleosides are limited to those which prevent transport of showdomycin into the cells. This conclusion is supported by experimental data from transport studies of showdomycin and nucleosides, primarily uridine, as summarized below. Firstly, uridine and showdomycin are mutually inhibitory for their transport in *E. coli* B cells (Tables II and VI). Secondly, the concentration of uridine required to prevent the inhibitory effect of showdomycin on glucose transport is approximately proportional to the concentration of the antibiotic (Table I). Thirdly, the transport of both showdomycin and uridine is markedly reduced in showdomycin-resistant mutants as compared to wild-type cells (Tables V and VI). Fourthly, uridine and showdomycin are not mutually inhibitory for their transport in mutant cells, in contrast to their effects in *E. coli* B (Tables V and VI). Thus, during the development of resistance towards showdomycin, that part of the transport process which is common to both showdomycin and uridine appears to have been lost.

The data describing the effects of unlabeled nucleosides on the transport of radioactive nucleosides (Table III) substantiate the above conclusions. With the exception of adenosine, nucleosides which protect against inhibition by showdomycin are mutually competitive for their transport, as would be expected if these nucleosides compete with showdomycin for a common transport process. The exceptional behavior of adenosine can be explained, as discussed later. In agreement with the above conclusions, pseudouridine, which does not inhibit transport of purine or pyrimidine nucleosides, is predictably not effective as a protective agent against showdomycin inhibition (Table III, ref. 3).

Guanosine, which does not protect cells from showdomycin inhibition or inhibit uptake of uridine or cytidine (Table III), appears to be transported by a process distinct from those nucleosides. Guanosine transport is inhibited strongly by adenosine (Table III), and adenosine transport is inhibited only slightly by guanosine. Similar results have been reported in mammalian cells by Plagemann *et al.*[10], who demonstrated that adenosine has a strong affinity for the guanosine transport in mammalian cells, whereas guanosine has relatively little effect on adenosine transport. A possible explanation, as proposed by these authors, is that adenosine has an affinity for the guanosine transport site, but requires a different site for its own transport.

A similar anomaly exists in the transport relationship between adenosine and pyrimidine nucleosides. Adenosine inhibits transport of uridine and cytidine, whereas these nucleosides do not inhibit adenosine transport (Table III). These results suggest that adenosine has a strong affinity for the uridine and cytidine transport site(s), but requires a separate site for at least some of its own transport. These interpretations are consistent with the observation that adenosine protects against the inhibitory effects of showdomycin, whereas guanosine, which does not inhibit transport of uridine and cytidine, is ineffective[3]. In agreement with the above explanation, 3′-deoxyadenosine (cordycepin), an analog of adenosine which does not compete with adenosine transport[8], does not reverse inhibition of glucose transport by showdomycin, whereas 2′-deoxyadenosine, which does compete for adenosine transport[8], is effective as a protective nucleoside[3]. Additional evidence that adenosine and guanosine transport is different from that of uridine, cytidine and showdomycin is apparent from the data of Table V. The transport capacities for adenosine and guanosine are similar in *E. coli* B and in showdomycin-resistant mutants, whereas uptake of cytidine, uridine and showdomycin is greatly reduced in mutant cells as compared to that in *E. coli* B (Tables V and VI).

Showdomycin inhibits transport of all nucleosides, including adenosine and guanosine (Table II). The inhibitory effect of showdomycin on uridine and cytidine transport may be explained by competition of these nucleosides for a common transport process. The inhibitory effect of showdomycin on adenosine and guanosine transport cannot be explained by competition for a common site. It is probable that showdomycin, like *N*-ethylmaleimide, reacts non-specifically with sulfhydryl groups of cellular enzymes or proteins, thus inactivating metabolic processes generally, including the transport of guanosine, adenosine, amino acids and sugars. Supportive evidence for this is provided by the data of Table V. In *E. coli* B cells the transport of guanosine and adenosine is inhibited similarly by showdomycin and *N*-ethylmaleimide but in mutant C the transport of these nucleosides is not inhibited by showdomycin, whereas sensitivity to *N*-ehtylmaleimide is retained as in *E. coli* B.

An explanation for the resistance of mutants to inhibition by showdomycin as well as the protective action of nucleosides in *E. coli* B is apparent from the data of Table VI. Transport of showdomycin is reduced to the same level in *E. coli* B in the presence of uridine as in mutant cells in its absence. It is of interest that uridine-protected *E. coli* B and the mutant have a high uptake of showdomycin, like that of the zero-time value (Table VI), even though both cell types are completely refractory to inhibition by exogenous showdomycin. These "blank" values are high, presumably because of chemical interaction of showdomycin with reactive groups at the cell surface which are not involved in transport. This assumption was supported by the

demonstration that glucose transport in *E. coli* B cells is unimpaired after pretreatment with showdomycin at o °C and immediate removal of unreacted showdomycin with excess β-mercaptoethanol.

It is apparent that the inability of exogenous showdomycin to inhibit the transport process of sugar in mutant cells results directly from loss of the mutant cell's capacity to transport the antibiotic (Table IV). Showdomycin-resistant mutants retain their sensitivity to *N*-ethylmaleimide inhibition of glucose and nucleoside uptake in a manner indistinguishable from *E. coli* B. Since inhibition of glucose uptake by showdomycin and *N*-ethylmaleimide in *E. coli* B occurs at similar concentration levels, and since both analogs exert their primary inhibitory effect by alkylation of susceptible sulfhydryl groups[3], it may be concluded that resistance to showdomycin inhibition by the mutants is the direct result of impairment of showdomycin transport into the cell rather than alteration of proteins reactive to these sulfhydryl reagents. It is probable that showdomycin exerts its inhibitory effects without prior conversion to the nucleotide level since it is not a substrate for nucleoside kinase or nucleoside phosphorylase in Ehrlich ascites cells[4]. Thus, a primary means available to the organism for development of resistance is the loss of its transport into the cell. It may be concluded that showdomycin exerts its inhibitory effects in the same non-specific manner as *N*-ethylmaleimide and other sulfhydryl reagents, but differs in that it has a requirement for a transport site for which the common nucleosides, uridine, cytidine and adenosine, compete.

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After submission of our manuscript we became aware of two publications by Komatsu[11,12] which demonstrate an inhibition of showdomycin transport by certain nucleosides in *E. coli* K-12 and a reduction in the transport of showdomycin and certain nucleosides in showdomycin-resistant *E. coli* K-12 mutants. Their conclusions are similar to several of those presented in this publication.

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REFERENCES

1 H. Nishimura and Y. Komatsu, *J. Antibiot.*, 21 (1968) 250.
2 Y. Komatsu and K. Tanaka, *Agric. Biol. Chem.*, 32 (1968) 1021.
3 S. Roy-Burman, Y. H. Huang and D. W. Visser, *Biochem. Biophys. Res. Commun.*, 42 (1971) 445.
4 S. Roy-Burman, P. Roy-Burman and D. W. Visser, *Cancer Res.*, 28 (1968) 1605.
5 K. R. Darnall, L. B. Townsend and R. K. Robins, *Proc. Natl. Acad. Sci. U.S.*, 57 (1966) 548.
6 M. Beljanski, P. Bourgarel and J. Chassagne, *C. R. Acad. Sci. Paris*, 269 (1969) 240.
7 B. D. Davis and E. S. Mingioli, *J. Bacteriol.*, 60 (1950) 17.
8 R. N. Peterson and A. L. Koch, *Biochim. Biophys. Acta*, 126 (1966) 129.
9 R. N. Peterson, J. Boniface and A. L. Koch, *Biochim. Biophys. Acta*, 135 (1967) 771.
10 P. G. W. Plagemann, *Biochim. Biophys. Acta*, 233 (1971) 688.
11 Y. Komatsu, *J. Antibiot.*, 24 (1971) 876.
12 Y. Komatsu, *Agric. Biol. Chem.*, 35 (1971) 1328.