MECHANISM OF ACTION OF SHOWDOMYCIN. V

REDUCED ABILITY OF SHOWDOMYCIN-RESISTANT MUTANTS OF *ESCHERICHIA COLI* K-12 TO TAKE UP SHOWDOMYCIN AND NUCLEOSIDES

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(Received for publication May 27, 1971)

I have isolated a number of mutants from *Escherichia coli* K-12 which are highly resistant to showdomycin [2-(β-D-ribofuranosyl)maleimide]. These mutants were very sensitive to N-ethylmaleimide, which has a structure similar to the aglycone moiety of showdomycin. These mutants took up little ^{14}C-labeled showdomycin and showed altered ability to take up various labeled nucleosides. However, their ability to take up cytosine arabinoside-^{3}H remained essentially the same as that of the parent strain. The ability of caffeine-treated mutant cells to take up adenosine-^{3}H was very much lower than that of caffeine-treated parent cells. The nature of deoxycytidine-^{14}C uptake by mutant cells was markedly different from that by parent cells.

The aglycone moiety of showdomycin (SHM) is maleimide[1,2,3]. Maleimide derivatives are known to be active alkylating agents for sulfhydryl, amino and imidazole groups[4,5,6,7], and direct and stoichiometric reaction of SHM with the thiol groups of mercaptoethanol[8], cysteine[9] and protein[10] have been reported.

The inhibitory effect of the antibiotic on the ribonucleotide reductase and dTMP synthetase systems in cell-free extracts of *E. coli*, an important evidence explaining the inhibition of DNA synthesis in the cells, may also be due to the alkylating function of the antibiotic[11].

The whole structure of SHM is a C-substituted nucleoside, closely related to pseudouridine (ψUr) and uridine (Ur)[1,9]. The inhibitory action of SHM *in vivo* is prevented by the addition of a wide variety of nucleosides[12,13,14,15].

In a preceding paper[16], the entry of ^{14}C-labeled SHM (SHM-^{14}C) into cells of *E. coli* was shown to be inhibited by a wide variety of nucleosides. The inhibition of SHM-^{14}C entry into the cells by adenosine (Ar) was competitive, suggesting that the two molecules share a common transport site.

The mechanism of the action of SHM may be explained by assuming that the antibiotic acts as a nucleoside analogue on entry into the cells but as a derivative of maleimide once it has entered.

A number of mutants of *Escherichia coli* K-12, highly resistant to SHM but sensitive to an analogue of the maleimide moiety of the antibiotic, N-ethylmaleimide (NEM), have been isolated.

Some observations on the physiological behavior of these mutants supporting the above hypothesis are reported here.

Materials and Methods

Isolation of SHM-resistant mutants: *E. coli* K-12 was incubated in a minimal medium[17] fortified with 0.1 % Bacto Yeast Extract and 0.1 % Bacto Casamino Acid (MYC medium) at 37°C with shaking until the cells were in logarithmic phase. A 30-ml sample of the culture was collected by centrifugation. The cells washed twice with 0.86 % saline solution and suspended in 18 ml of pH 6.1 Tris-maleate buffer[18] (5.2×10^8 cells/ml) and N-methyl-N'-nitro-N-nitrosoguanidine (NG) added directly to the suspension in a final concentration of 102 μg/ml. After incubation at 37°C for 15 minutes, cells were collected, washed twice with saline solution and resuspended in 30 ml of MYC medium. Survival was 23 %. The cells were divided among 10 tubes and incubated at 37°C for 19 hours. The cultures (survival cell concentration $1.30\sim1.32\times10^9$ cells/ml) were diluted with saline solution to $6.1\sim6.3\times10^7$ cells/ml and 0.5-ml aliquots plated onto MYC medium agar plates containing 400 μg SHM per ml. Colonies growing on the plates were arbitrarily isolated, the yields of SHM-resistant mutants ranging from 5.5 to 18.2 per 10^6 of survivors. The isolated mutants were sensitive to NEM, erythromycin, streptomycin and chloramphenicol.

Incorporation of uracil-^{14}C and lysine-^{3}H into *E. coli*: An *E. coli* cell suspension prepared as reported previously[14] was incubated with 15 μM of uracil-2-^{14}C (0.67 μCi/μmole), 10 μM of lysine-U-^{3}H (50 μCi/μmole) and SHM or NEM (Table 1) at 37°C for 20 minutes. The incorporation of these radioactive precursors was measured by precipitation of protein and nucleic acids with TCA 5 % cold. The precipitates were collected on glass fiber paper (Whatman GF/C), washed with ether-ethanol solution (2 : 1) and counted in a liquid scintillation spectrometer using toluene phosphor solution.

Incorporation of SHM-^{14}C and other labeled nucleosides by *E. coli* cells: The uptake of SHM-^{14}C and other labeled nucleosides was measured by rapid chilling and rapid Millipore filtration techniques as previously described[16].

Chemicals and reagents: SHM-^{14}C was obtained by incubation of *Streptomyces showdoensis* with sodium acetate-2-^{14}C[20]; NG was purchased from the Aldrich Chemical Co., NEM from Nakarai Chemicals Ltd., the labeled compounds from Schwarz BioReseach Corp., unlabeled nucleosides from Sigma Chemical Co. and Schwarz BioResearch Corp.

Results

Susceptility of SHM-Resistant Mutants to NEM

If the SHM-resistance of a mutant is due to its insusceptibility to the alkylating action of the antibiotic, the mutant should also be resistant to NEM, an analogue of the maleimide moiety of SHM. To investigate this, the effects of SHM and NEM on the incorporation of uracil-^{14}C and lysine-^{3}H into the nucleic acids and protein of SHM-resistant mutant (Shm^r-001) cells was compared with that in parent cells. The results shown in Table 1 demonstrated that the ability of the mutant to incorporate these labeled precursors was nearly the same as that of parent cells, and that their incorporation was not affected appreciably by SHM but inhibited by NEM.

A sample of SHM-resistant colony was inoculated onto an MYC medium agar containing 100 μg/ml NEM, using sterile toothpicks. Growth was not observed among the 1,010 samples tested.

Similarly, suspensions of NG-treated cells, containing 1.32×10^{10} survival cells or 1.56×10^5 SHM-resistant mutant cells, were plated on the MYC medium agar containing 100 μg/ml NEM, no colony was observed after 3 days incubation at 37°C.

These results would indicate that the SHM-resistant mutants obtained are susceptible to the alkylating action of SHM.

Reduced Ability of SHM-Resistant Mutants to Take up SHM-^{14}C

Another possible mechanism to be considered for the mutation to SHM-resistance is a loss of the ability to transport SHM into the cells. As shown in Table 2, the uptake of SHM-^{14}C by the mutants was markedly less than that of the parent strain, suggesting that the mutational changes in the susceptibility to SHM may involve a reduced ability of SHM to penetrate the cell membrane.

Reduced Ability of the Mutants to Take up Labeled Nucleosides

A close relationship observed between the transport of SHM-^{14}C and nucleosides[16] led to consideration of uptake of labeled nucleosides by the mutants as compared with the parent strain.

The uptake of adenosine-^{3}H (Ar-^{3}H), deoxycytidine-^{14}C (Cdr-^{14}C), cytidine-^{3}H (Cr-^{3}H) and deoxyguanosine-^{14}C (Gdr-^{14}C) by the mutants was compared with that by the parent strain during incubation at 37°C for 3 minutes. As shown in Table 3, the mutants showed reduced uptake of Cdr-^{14}C and Cr-^{3}H, while Ar-^{3}H and Gdr-^{14}C uptake remained essentially the same as in the parent strain. Fig. 1 shows the typical time course of uptake of Cdr-^{14}C and Ar-^{3}H by the mutant and parent strains during incubation at 37°C. A marked difference is seen in the time-dependent uptake of Cdr-^{14}C between the mutant and parent strains but little difference in Ar-^{3}H uptake between these two strains; uptake of Ar-^{3}H by the mutant is as rapid as the parent even after 30 seconds at 37°C. At this temperature, the nucleoside uptake was too rapid for careful measurement to be made by the present method. At a lower incubation temperature, however, the rate of nucleo-

Table 1. Effect of SHM and NEM on the incorporation of uracil-^{14}C and lysine-^{3}H in parent and mutant cells

Reagent (μM)		Uracil-^{14} uptake (cpm)*		Lysine-^{3}H uptake (cpm)*	
		Parent	Shm^{r}-001	Parent	Shm^{r}-001
None		3,832	3,549	28,154	28,229
SHM	2.4	2,327	2,907	20,703	22,569
	4.8	890	3,154	5,864	24,091
	9.6	428	2,794	2,941	22,158
	28.8	221	2,714	1,175	21,756
NEM	2.4	3,201	2,719	24,212	21,988
	4.8	2,519	2,413	22,457	20,819
	9.6	823	1,237	7,921	16,234
	43.5	505	247	1,274	1,049

Parent and mutant cells were incubated with uracil-^{14}C, lysine-^{3}H and cited amounts of SHM or NEM at 37°C for 20 minutes. For further explanation see text.
* cpm/ml cells (O.D.$_{660}$=0.1)/20 min. at 37°C

Table 2. Reduced ability of SHM-resistant mutants cells to take up SHM-^{14}C

Experiment	Strain	SHM-^{14}C uptake*
I	Parent	0.558
	Shm^{r}-001	0.022
	Shm^{r}-007	0.041
II	Parent	0.554
	Shm^{r}-025	0.018
	Shm^{r}-031	0.030
	Shm^{r}-037	0.031
	Shm^{r}-043	0.031
	Shm^{r}-049	0.056
	Shm^{r}-055	0.029
III	Parent	0.478
	Shm^{r}-063	0.073
	Shm^{r}-071	0.110
	Shm^{r}-075	0.065
	Shm^{r}-079	0.054

After the cell suspensions of parent and SHM-resistant mutants (O.D.$_{660}$=0.162~0.189 in experiment I, 0.332~0.389 in experiment II and 0.262~0.332 in experiment III) had been allowed to equilibrate for 6 minutes at 37°C, one ml samples were added to tubes containing 7,652 cpm SHM-^{14}C (11.6 μM final concentration), and incubated at 37°C for 5 minutes (in experiment I) or 3 minutes (in experiments II and III). The cells were collected on a Millipore filter, washed, and the total radioactivity of the cells was determined.
* nanomoles/ml cells (O.D.$_{660}$=0.1)

Table 3. Reduced ability of SHM-resistant mutants to take up Cdr-^{14}C and Cr-^{3}H uptake of labeled nucleoside (nanomole*)

Labeled nucleoside	Experiment I			Experiment II				
	Parent	Shm^{r}-001	Shm^{r}-007	Parent	Shm^{r}-063	Shm^{r}-071	Shm^{r}-075	Shm^{r}-079
Cdr-^{14}C	1.34	0.57	0.79	1.63	0.54	0.58	0.56	0.59
Cr-^{3}H	0.97	0.47	0.74	1.00	0.66	0.52	0.61	0.54
Ar-^{3}H	0.62	0.72	0.89	0.92	1.52	0.92	1.13	0.90
Gdr-^{14}C	0.91	0.88	0.96	0.86	1.10	1.10	1.00	0.80

After the cell suspensions of parent (O.D.$_{660}$=0.348 in experiment I and 0.318 in experiment II), Shm^{r}-001 (O.D.$_{660}$=0.345), Shm^{r}-007 (O.D.$_{660}$=0.240), Shm^{r}-063 (O.D.$_{660}$=0.313), Shm^{r}-071 (O.D.$_{660}$=0.291), Shm^{r}-075 (O.D.$_{660}$=0.262) and Shm^{r}-079 (O.D.$_{660}$=0.332) had been allowed to equilibratef for 6 minutes at 37℃, one ml samples were added to tubes containing two of labeled nucleosides (each 10 μM final concentration), and incubated at 37℃ for 3 minutes. The Cdr-^{14}C uptake was measured in the presence of Ar-^{3}H. The Cr-^{3}H uptake was measured in the presence of Gdr-^{14}C.

* nanomoles/ml cells (O.D.$_{660}$=0.1)/3 minutes at 37℃.

Abbreviation: Cdr-^{14}C, deoxycytidine-2-^{14}C; Cr-^{3}H, cytidine-5-^{3}H; Ar-^{3}H, adenosine-8-^{3}H; Gdr-^{14}C, deoxyguanosine-8-^{14}C.

Fig. 1. Time courses of the uptake of Cdr-^{14}C and Ar-^{3}H by parent and mutant cells during incubation at 37℃.

Cell suspensions of parent (O.D.$_{660}$=0·289) and SHM-resistant mutant Shm^{r}-001 (O.D.$_{660}$=0.313) were incubated with 11,250 cpm Cdr-^{14}C (10 μM final concentration) and 207,150 cpm Ar-^{3}H (10 μM final concentration) at 37℃. One ml aliquots were removed after the indicated periods and prepared for counting as described in Materials and Methods.

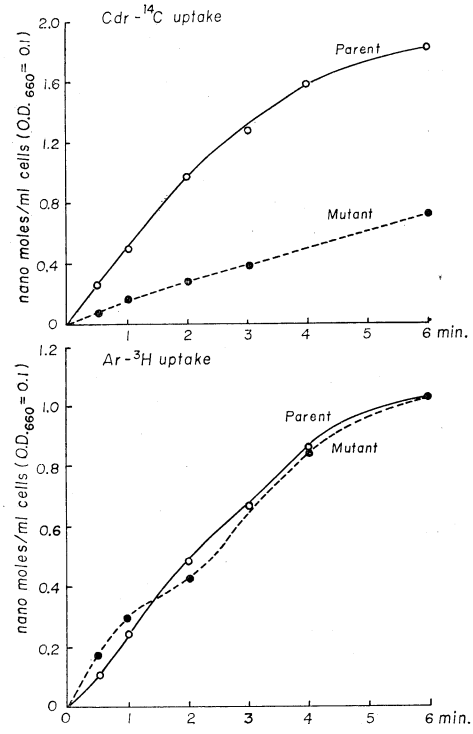


Table 4. Ar-^{3}H and Cdr-^{14}C uptake by mutant and parent cells at lower temperatures

Labeled nucleoside	Strain	Uptake of labeled nucleosides (nmole*)		
		at 7℃	at 17℃	at 27℃
Cdr-^{14}C	Parent	0.090	0.253	0.555
	Shm^{r}-001	0.019	0.081	0.216
Ar-^{3}H	Parent	0.136	0.190	0.379
	Shm^{r}-001	0.051	0.150	0.369

After the cell suspensions of parent (O.D.$_{660}$=0.289) and SHM-resistant mutant Shm^{r}-001 (O.D.$_{660}$=0.313) had been allowed to equilibrate for 6 minutes at the desired temperature, one ml samples were added to tubes containing 11,250 cpm Cdr-^{14}C (10 μM final concentration) and 207,150 Ar-^{3}H (10 μM final concentration), and incubated at the respective temperature for 3 minutes. The cells were collected on a Millipore filter, washed, and the total radioactivity of cells was determined.

* nanomoles/ml cells (O.D.$_{660}$=0.1)/3 minutes.

side uptake decreased so that the difference in uptake between mutant and parent cells could be examined more precisely. As shown in Table 4, it was found that at lower temperatures not only the Cdr-^{14}C but also the Ar-^{3}H uptake by the mutant was significantly less than that of the parent strain.

The uptakes of various labeled nucleosides by the mutant and parent strain were compared during incubation at 0°C. The results summarized in Table 5 indicate that at 0°C uptake of a wide variety of common nucleosides by the mutant was significantly less than that of the parent strain, suggesting that the transport system of the former was altered in mutation. It is of interest to note that among the cytosine nucleosides, uptake of cytosine riboside (Cr-^{3}H) and cytosine deoxyriboside (Cdr-^{14}C)

Table 5. Reduced ability of the SHM-resistant mutant to take up various nucleosides at 0°C

Labeled nucleoside	Uptake* at 0°C		Uptake* at 37°C	
	Parent	Shm^r-001	Parent	Shm^r-001
SHM-^{14}C	0.080	0.009	0.54	0.04
Ar-^{3}H	0.104	0.031	0.87	0.84
Adr-^{14}C	0.179	0.044	1.32	1.33
Gdr-^{14}C	0.070	0.044	1.00	0.86
Ur-^{14}C	0.095	0.048	1.46	0.84
Cr-^{3}H	0.070	0.022	1.15	0.60
Cdr-^{14}C	0.085	0.031	1.57	0.89
Tdr-^{14}C	0.029	0.008	0.073	0.051
CAra-^{3}H	0.028	0.023	0.151	0.130
U-^{14}C	0.196	0.261	1.64	2.37

After the cell suspensions of parent (O.D.$_{660}$=0.201) and SHM-resistant mutant Shm^r-001 (O.D.$_{660}$=0.227) had been allowed to equilibrate for 6 minutes at the desired temperature, one ml samples were added to tubes containing labeled nucleosides (each 10 μM final concentration), and incubated for 3 minutes at 37°C or 6 minutes at 0°C.
* nanomoles/ml cells (O.D.$_{660}$=0.1)/3 minutes (at 37°C) or 6 minutes (at 0°C).
Abbreviation: Adr-^{14}C, deoxyadenosine-8-^{14}C; Ur-^{14}C, uridine-2-^{14}C; Tdr-^{14}C, deoxythymidine-2-^{14}C; CAra-^{3}H, cytosine arabinoside-5-^{3}H; U-^{14}C, uracil-2-^{14}C.

Table 6. Ar-H^3 uptake by mutant and parent cells in the presence and absence of caffeine

Caffeine added (mM)	Ar-^{3}H uptake (nanomole*)	
	Parent	Shm^r-001
0	0.51	0.62
9	0.181	0.063

After the cell suspensions of parent (O.D.$_{660}$=0.247) and SHM-resistant mutant Shm^r-001 (O.D.$_{660}$=0.281) had been allowed to equilibrate for 6 minutes at 37°C, one ml samples were added to tubes containing 214,670 cpm Ar-^{3}H (10 μM final concentration) and with or without caffeine, and incubated at 37°C for 3 minutes. The cells were collected on a Millipore filter, washed, and the total radioactivity of cells was determined.
* nanomoles/ml cells (O.D.$_{660}$=0.1)/3 minutes at 37°C.

of the mutant cells was markedly reduced, whereas that of cytosine arabinoside (CAra-^{3}H) remained almost intact; uptake of uracil-^{14}C (U-^{14}C) by the mutant was increased, while that of uridine-^{14}C (Ur-^{14}C) was markedly reduced.

The most important evidence relating to the ability of the mutant to transport nucleoside has been obtained with caffeine-treated cells. PETERSON *et al.*[19] reported that in caffeine-treated cells incubated with Ar, the entry and deamination of Ar proceeded essentially at a normal rate, however subsequent metabolism and efflux of inosine were not observed. To study Ar-^{3}H transport separately from its subsequent metabolism, uptake of the nucleoside by the mutant cells was compared with parent cells in the presence of caffeine. The results revealed that the Ar-^{3}H uptake by the caffeine-treated mutant cells was very much lower than that of similarly-treated parent cells (Table 6), suggesting a reduced ability of the cell membrane of the mutant to transport Ar-^{3}H into the cells.

The fact that the mutant cells showed reduced uptake of SHM as well as diminished transport of nucleosides strongly indicates that there is a close relationship between the transport systems for SHM and nucleosides in *E. coli* cells.

Nature of Cdr-^{14}C Uptake by an SHM-Resistant Mutant

At a higher temperature, the mutant cells accumulated a significant quantity of radioactivity when incubated with labeled nucleosides, although showing reduced uptake of the same nucleosides at lower temperatures. To determine if different transport mechanisms operate in parent and mutant cells, the Cdr-^{14}C uptake were compared.

First, the effect of various nucleosides on Cdr-^{14}C uptake at 37°C was compared in mutant and parent cells. As shown in Table 7, the uptake of Cdr-^{14}C by parent cells was increased by the addition of low concentrations of other nucleosides [with the exception of Cr, Ur, deoxyuridine (Udr) and SHM]; similar treatments with mutant cells decreased the uptake [with the exception of xanthosine (Xr), ψUr, CAra

and SHM]. Figs. 2 and 3 give the results of more detailed study on the Cdr-^{14}C uptake. Fig. 2 shows the Cdr-^{14}C uptake by parent cells susceptible to SHM. Under similar conditions, the mutant was highly resistant. Fig. 3 shows a marked difference between mutant and parent cells to the effect of Ar on the Cdr-^{14}C uptake. The Cdr-^{14}C uptake by parent cells was slightly stimulated by low concentrations of Ar, whereas similar concentrations markedly inhibited uptake by mutant cells.

Next, the effect of different external concentrations (over a 31-fold range) of Cdr-^{14}C on its uptake by mutant and parent cells was examined (Fig. 4). It is of interest to note that the rate of Cdr-^{14}C uptake by parent cells remained essentially constant over a wide range of Cdr concentrations, while that by mutant cells increased with increasing external concentrations of Cdr. At lower concentrations, Cdr was taken up by mutant

Table 7. Effect of various nucleosides on the Cdr-^{14}C uptake by mutant and parent cells

Nucleoside added (each 9.8 μM)	Cdr-^{14}C uptake*	
	Parent	Shm^r-001
None	1.24	0.88
Adenosine	1.50	0.59
Deoxyadenosine	1.31	0.77
Deoxyguanosine	1.58	0.67
Guanosine	1.62	0.66
Inosine	1.59	0.64
Cytidine	0.66	0.62
Uridine	0.72	0.65
Deoxyuridine	0.64	0.61
5-Iodouridine	1.45	0.54
5-Bromodeoxyuridine	1.48	0.37
Deoxythymidine	1.26	0.68
Xanthosine	1.44	0.88
Pseudouridine	1.38	0.84
Cytosine arabinoside	1.32	0.88
Showdomycin	0.96	0.87

After the cell suspensions of parent (O.D.$_{660}$=0.312) and SHM-resistant mutant Shm^r-001 (O.D.$_{660}$=0.338) had been allowed to equilibrate for 6 minutes at 37℃, one ml samples were added to tubes containing 11,250 cpm Cdr-^{14}C (9.8 μM final concentration) and one of various nucleosides (each 9.8 μM final concentration), and incubated at 37℃ for 3 minutes.
* nanomoles/ml cells (O.D.$_{660}$=0.1)/3 minutes at 37℃.

Fig. 2. Effect of SHM on the uptake of Cdr-^{14}C by parent and mutant cells.

After the cell suspensions of parent (O.D.$_{660}$=0.310) and SHM-resistant mutant Shm^r-001 (O.D.$_{660}$=0.331) had been allowed to equilibrate for 6 minutes at 37℃, one ml samples were added to tubes containing 11,250 cpm Cdr-^{14}C (10 μM final concentration) and the cited amount of SHM, and incubated at 37℃ for 3 minutes. The cells were collected on a Millipore filter, washed, and the total radioactivity of cells was determined.

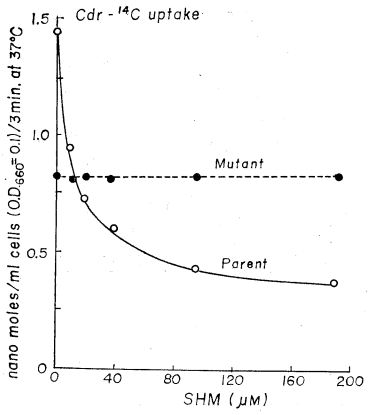
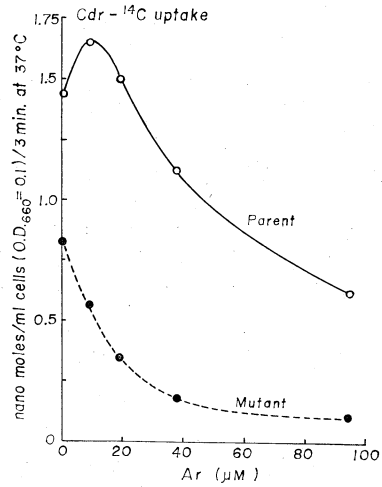


Fig. 3. Effect of Ar on the uptake of Cdr-^{14}C by parent and mutant cells.

After the cell suspensions of parent (O.D.$_{660}$=0.310) and SHM-resistant mutant Shm^r-001 (O.D.$_{660}$=0.331) had been allowed to equilibrate for 6 minutes at 37℃, one ml samples were added to tubes containing cpm Cdr-^{14}C (10 μM final concentration) and the cited amount of Ar, and 11,250 incubated at 37℃ for 3 minutes. The cells were collected on a Millipore filter, washed, and the total radioactivity of cells was determined.

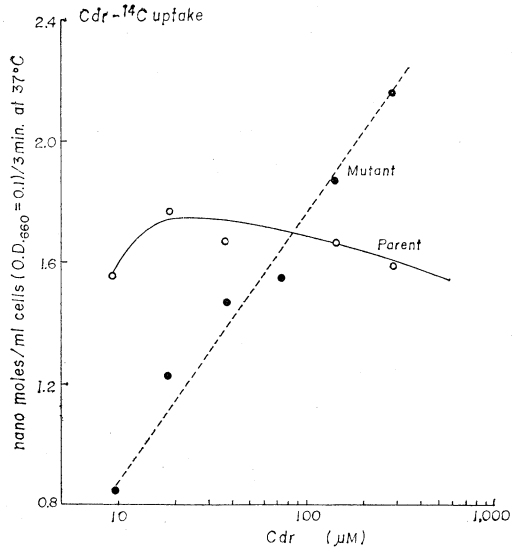


cells at a slower rate than by parent cells, whereas at high concentrations the uptake was more rapid.

It was concluded that the nature of Cdr-^{14}C uptake by mutant cells is markedly different from parent strain.

Fig. 4. Effect of different external concentrations of Cdr on its uptake by mutant and parent cells.

After the cell suspensions of parent (O. D. $_{660}$=0.279) and SHM-resistant mutant Shm^r-001 (O. D. $_{660}$=0.307) had been allowed to equilibrate for 6 minutes at 37℃, one ml samples were added to tubes containing cited amounts of Cdr-^{14}C (over a 31-fold range), and incubated at 37℃ for 3 minutes. The cells were collected on a Millipore filter, washed, and the total radioactivity of cells was determined.



Discussion

A number of *Escherichia coli* K-12 mutants have been isolated which are highly resistant to SHM, and highly sensitive to NEM.

As previously reported, the inhibitory action of SHM *in vivo* was cancelled by the addition of nucleosides, while that of NEM was not reversed. This indicates a marked difference in the mechanism of action of SHM and NEM, despite their similar action in cell-free systems as alkylating agents. This difference may be due to a difference in the transport system for SHM and NEM. Since uracil-^{14}C and lysine-^{3}H incorporation in the SHM-resistant mutant cells was strongly inhibited at low concentrations of NEM (Table 1), the ability of the mutants to transport the reagent apparently remained the same as in the parent strain. In contrast to this, the marked reduction of SHM-^{14}C uptake by the mutant cells suggests that the transport system for SHM was altered by the mutation.

The transport system for SHM in *E. coli* appears to be closely related to that for the transport of a wide variety of nucleosides. In another paper[16], it was shown that the entry of SHM-^{14}C into parent cells is inhibited by the addition of nucleosides. In the present study, it was found that the mutants show a marked reduction in nucleoside uptake paralleling reduced SHM-^{14}C uptake. The results obtained on the uptake of labeled nucleosides at 0°C (Table 5) and Ar-^{3}H uptake by caffeine-treated cells (Table 6) clearly suggest that the reduction of nucleoside uptake is due to a mutational alteration of transport mechanism of the cell membrane. This functional alteration could be located at an outer site of the cell membrane, since inhibition of SHM-^{14}C uptake by nucleoside would normally occur at an outer site.

The site involved in CAra transport, however, appears to be independent of the SHM transport site as the mutant defective in SHM-^{14}C uptake was as capable as the parent strain in CAra-3H (Table 5) suggesting that the CAra transport mechanism has remained intact.

The physiological implications of the system involved in SHM transport have yet to be investigated. It is noted, however, that an SHM-resistant mutant could grow very well on a minimal medium and that it incorporated uracil and lysine at the same rate as the parent strain.

Acknowledgement

The author is indebted to Dr. KENTARO TANAKA for his helpful advice and interest in this work.

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