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ENERGY REQUIREMENTS, INTERACTIONS AND DISTINCTIONS IN THE MECHANISMS FOR TRANSPORT OF VARIOUS NUCLEOSIDES IN *ESCHERICHIA COLI*

R. N. PETERSON*, J. BONIFACE AND ARTHUR L. KOCH
Department of Biochemistry, University of Florida College of Medicine, Gainesville, Fla. (U.S.A.)

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SUMMARY

The transport of cytidine and uridine in *Escherichia coli*, like that of adenosine, is sensitive to energy poisons and apparently can be coupled to energy metabolism. The depletion of energy reserves, however, does not prevent the passive uptake of cytidine which indicates that an active pumping mechanism is not an essential or obligatory component of the transport process. This finding, together with similar results previously reported for adenosine and β-galactoside transport, suggests that energy coupling to non-electrolyte transport generally may be facultative in *E. coli*.

Adenosine, cytidine and uridine strongly compete with one another for entry. However, the three transport sites are not identical since each site shows a different sensitivity to the action of thiol reagents and exposure to high temperatures.

Cytidine transport sites are only partially destroyed by treatment with large concentrations of *N*-ethylmaleimide, suggesting that more than one kind of site mediates the entry of this nucleoside. The sensitive portion has a reactivity to *N*-ethylmaleimide comparable to those sites for adenosine and uridine.

Both adenosine and uridine inhibit cytidine transport with competitive kinetics in untreated cells. In cells in which the transport sites of the competing nucleosides have been abolished by treatment with thiol reagents together with heat, cytidine transport is still inhibited.

Possible mechanisms to explain these interactions are discussed.

INTRODUCTION

In a previous communication[1] it was shown that the purine nucleosides, adenosine and inosine, are transported into cells of *Escherichia coli* by a stereospecific mechanism that requires the intact riboside structure and which is facultatively coupled to energy metabolism. The observations that nucleosides generally competed

Abbreviation: PCMB, *p*-chloromercuribenzoate.

* Present address: Department of Pharmacology, New York Medical College, New York, N.Y. (U.S.A.)

with these two nucleosides for entry and that the presence of exogenous nucleosides speeded the efflux of accumulated inosine suggested the presence of a common carrier transport element. The presence of a second, and perhaps more stereospecific element was indicated by the fact that the methylxanthine, caffeine, specifically inhibited both inosine entry and efflux but had little or no effect on the entry rates of other nucleosides.

We have now extended the studies to include the transport of pyrimidine nucleosides, cytidine and uridine. It has been possible to discern further evidence for a common element and also specific differences in the mechanisms by which individual nucleosides enter cells. These results strengthen the conclusion that selective but interacting mechanisms function in the transport of nucleosides.

METHODS

The techniques and procedures previously described were employed[1-4].

Culture of organisms

E. coli strain B were harvested in logarithmic growth phase from liquid medium M-9. The cells were treated with chloramphenicol (10–50 μg/ml) and chilled until used. In some instances glucose or glycerol (1%) was used as carbon source; but for most experiments, cells were adapted to grow on uridine (0.1%) as sole source of carbon. After several daily transfers, generation times in uridine approximated that obtained in glucose supplemented medium (approx. 60 min). The high levels of cytidine deaminase (cytidine → uridine) and uridine phosphorylase (uridine → uracil) present in these cells (10-fold greater than unadapted cells) greatly extended the range of concentrations of substrates and inhibitors over which the spectrophotometric assays could be employed.

Results are presented in this paper on a mg dry wt. basis instead of per cell. Under the growth conditions employed where the doubling time is 50–60 min, there are 8.2×10^8 cells per mg dry wt. This factor allows comparison with our previous results[1,2].

Spectrophotometric assay

Steady-state cellular uptake of the nucleosides, followed by metabolism and release of products from the cell, were monitored by the absorbance changes of whole cell suspensions in the ultraviolet. Adenosine is hydrolytically deaminated to inosine; cytidine is hydrolytically deaminated to uridine. Both products appear in the external medium. Uridine is also taken up by cells and undergoes enzymatic reactions giving rise to the formation of uracil, which then appears in the suspending medium. Table I shows the changes in molar extinction coefficients at appropriate wavelengths in the ultraviolet that accompany these processes. Measurements of cellular cytidine deamination were made at 235 mμ instead of at 280 mμ where there is also a large change in absorbance, since at the former wavelength the kinetics are not obscured by the subsequent rapid cleavage of uridine. The uridine cleavage reaction measured at 270 mμ is not complicated by the metabolism of the reaction product, uracil, since the latter is taken up by cells at a 20- to 50-fold slower rate. All measurements were made directly on cell suspensions or cell lysates in a thermostatted Gilford recording

TABLE I

CHANGES IN MOLAR EXTINCTION COEFFICIENTS ACCOMPANYING DEAMINATION AND CLEAVAGE REACTIONS

Glucose medium M-9, pH 7.0.

Reaction	*Monitored wavelength (mμ)*	$\Delta\varepsilon \times 10^{-3}$
Adenosine → inosine	267	7.08*
Cytidine → uridine	235	5.31**
Uridine → uracil	270	2.83**

* Data from MANS AND KOCH[2].

** Obtained from absorbance measurements of high-purity commercial materials. Solutions were standardized spectrophotometrically at pH 2.0 (extinction coefficients taken from published values of VOLKIN AND COHN[5]).

spectrophotometer at 28°. Cell-free extracts were prepared by rupture of cells with ultrasonic radiation[1]. These were then clarified by centrifugation. At saturating substrate concentrations cytidine deamination gives rise to essentially linear changes in absorbance, as we have previously found for adenosine deamination. Uridine cleavage, however, is characterized by two kinetic processes. There is an initial and rapid change in absorbance followed by a second and slower change. The latter rate is also linear and is responsible for the consumption of the major portion of added substrate. This kinetic profile is observed with both intact cells and cell-free enzyme extracts. Whether these changes are due to the presence of two separate enzymatic reactions or to some form of enzyme inhibition must still be determined. For the purposes of these experiments, however, initial slopes were used to calculate reaction rates. We estimate that all rates reported here are accurate to at least 5%.

Isotopic measurements

The initial rates of entry and the accumulation of isotopic nucleosides were measured by the technique of rapid chilling and rapid Millipore filtration as previously described[1,3].

MATERIALS

Adenosine, cytidine and uridine were purchased from Calbiochem. Other nucleosides were purchased from Cyclo Chemical Corporation and the Sigma Chemical Company. [2-^{14}C]Uridine was purchased from Schwartz Bio Research Company. A paper chromatogram of a 0.1-μC sample of the compound was scanned with a Forro strip counter and found to be at least 99% radiochemically pure.

RESULTS

Energetics-stereospecificity

Double reciprocal plots of the kinetics of the cellular and cell-free cytidine deamination and uridine cleavage are shown in Fig. 1. It will be noted that the apparent binding constants for both cellular reactions are 10-fold lower than those

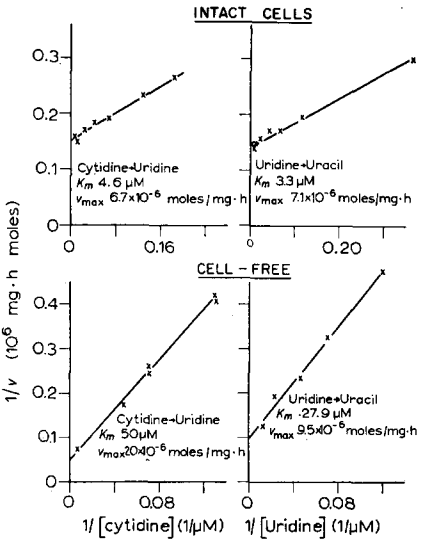


Fig. 1. Lineweaver–Burk plots of cellular and cell-free deamination and cleavage reactions for cells adapted to grow on uridine as sole carbon source. The cell suspensions contained 10 μg/ml chloramphenicol.

obtained for the cell-free preparations. If it is assumed that the binding constants for the enzymes *in vivo* are not changed under conditions *in vitro*, then these results suggest that there is a transport pump capable of saturating the internally located enzymes at external concentrations well below the respective values of K_m for the enzymes.

A similar conclusion can be drawn from the effects of energy poison. Cellular cytidine deamination, particularly at low substrate concentrations, is inhibited by sodium azide although cell-free extracts are not affected by this agent. Azide, at 50 mM, reduced the rate of cellular cytidine deamination to 75% of uninhibited controls at 10 μM cytidine, but only 26% of controls at 200 μM substrate concentration in uridine adapted cells. In such cells there is an apparent 3-fold excess of enzyme as estimated by the maximum rate of hydrolysis of the supernatants of sonicates to the maximum rate of hydrolysis by whole cells (see Fig. 1 or Table II). In glycerol grown

TABLE II

KINETIC CONSTANTS FOR CYTIDINE DEAMINATION BY INTACT CELLS AND CELL-FREE ENZYME EXTRACTS

The values are given as an average of several independent experiments.

	K_m (μM)		v_{max} (μmole/mg·h)	
	Uridine-grown cells	*Glycerol-grown cells*	*Uridine-grown cells*	*Glycerol-grown cells*
Cell-free enzyme	42.0	41.0	15.0	0.47
Intact cells	4.1	3.7	6.2	0.47
Intact cells + 50 mM azide	39.4	33.7	4.7	0.24

cells, where no apparent excess of enzyme could be demonstrated, inhibition by azide was more marked and was 85% and 50% at the low and high concentrations, respectively, implying that metabolic coupling is more essential than when there is an excess of enzyme. With cells grown on both carbon sources, the changes in rate are the result of an increase in the apparent K_m for the cellular reaction to values almost identical to that of the cell-free enzyme. These observations indicate that internal enzyme levels are apparently limiting the rate of cellular deamination; but more importantly, it also means that any active transport mechanism is not an obligatory

TABLE III

EFFECT OF NUCLEOSIDES, NUCLEOTIDES, FREE BASES AND CARBOHYDRATES ON CYTIDINE DEAMINATION BY INTACT CELLS

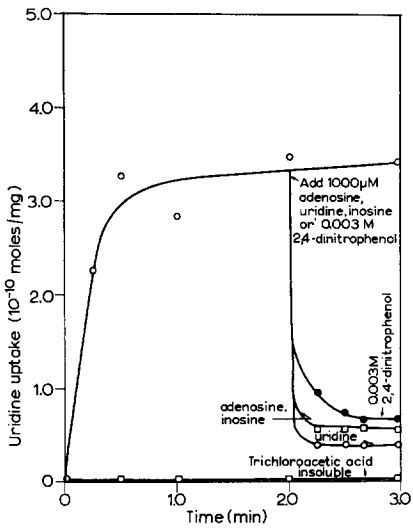
A. Substrate concn. 20 μM, competitor 200 μM. The control rate corresponded to an absorbance change of 0.0148 unit/min or to 6.6 μmoles cytidine deaminated/mg·h cell dry wt. B. Substrate concentration 100 μM, competitor 1700 μM. The control rate corresponded to an absorbance change of 0.0645 unit/min or to 6.7 μmoles cytidine deaminated/mg·h cell dry wt.

	Competitor	*Inhibition (%)*
A	Control	[0]
	Adenosine	93.3
	Inosine	49.7
	Guanosine	33.8
	Uridine	71.8
	Thymidine	83.0
	6-Chloropurine ribonucleoside	67.4
	Purine ribonucleoside	67.4
	Deoxycytidylic acid	4.9
	Cytosine	10.1
	Uracil	4.9
	Adenine	−1.3
B	Control	[0]
	Glucose	0.3
	Ribose	0
	Lactose	0.8

component of the nucleoside permeation process (*i.e.* the transport mechanism can also passively mediate nucleoside entry). Similar observations of these kinds have been described for adenosine transport[1] and apply as well to the galactoside[3] uptake mechanism; this admits the possibility that energy coupling may be generally facultative in microorganisms.

Cellular cytidine deamination is inhibited by other nucleosides but is not affected by free nucleic acid bases or very large amounts of glucose, ribose or lactose (Table III). This again implies that the transport system is unique for nucleosides and does not overlap mechanisms for the transport of other types of compounds. Uridine uptake and cleavage is similarly refractory to nucleic acid bases; in particular, the reaction is not inhibited by large concentrations of uracil. However, when cells are grown in the presence of glucose, even briefly, glucose, α-methylglucoside and 2-deoxyglucose inhibit the rate of uridine cleavage. Whether this behavior is related to transport or to some other aspect of glucose metabolism is still to be determined.

The rapid further metabolism and efflux of cytidine and uridine have precluded any direct measurements of the ability of intact, normal cells to accumulate pyrimidine nucleosides or to exchange internal pools with extracellular transport substrates. Such a difficulty can be partly overcome by using reagents that can block complicating metabolic events that occur subsequent to transport. In our previous studies of the adenosine transport mechanism[1,4], caffeine was useful for these purposes since the compound blocked adenosine incorporation into phosphorylated acid-soluble intermediates and nucleic acids, but did not affect the transport mechanism. Caffeine does not act on pyrimidine nucleoside transport or metabolism, but we have found that *N*-ethylmaleimide can effectively block the incorporation of nucleosides into nucleic acids. In addition, conditions can be found where the transport mechanism is



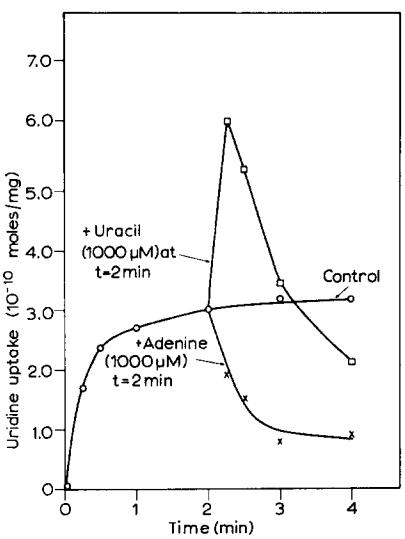


Fig. 2. Uptake of [2-^{14}C]uridine into cells pretreated with 5 mM *N*-ethylmaleimide at 28° for 15 min. Reaction tubes contained 5.2 μM [2-^{14}C]uridine (39 μC/μmole), glycerol (0.1%) and the cell suspension equivalent to 0.171 mg dry wt. of cells. Separate aliquots of the cell suspension were used to follow uptake over each time interval. Zero-time controls were taken by injecting an aliquot of ice-cold cells into ice-cold radioactive medium and rapidly filtering the suspension. The plateau level of accumulation (1700 counts/min) represented 1.67% of the total amount of radioactivity added (163 000 counts/min). The zero time controls were approx. 5% of the total radioactivity accumulated, and these values have been subtracted from all the data.

Fig. 3. The effect of the addition of non-radioactive adenine and uracil on the plateau level of accumulation of [2-^{14}C]uridine by cells pretreated with 5 mM *N*-ethylmaleimide. Conditions were the same as those indicated in Fig. 2.

only partially inactivated while further metabolism is almost completely blocked. In Fig. 2 the uptake of [2-^{14}C]uridine has been followed into both the acid-soluble fraction and the acid-insoluble fraction of cells after treatment with 5 mM *N*-ethylmaleimide. Although total uptake is considerably reduced after treatment (the rate of uridine cleavage is reduced to 25% of controls—see Fig. 6), the cells are still capable of accumulating a significant amount of radioactivity. In less than a minute the pool of radioactive material accumulated in this manner reaches a steady state.

The majority of this pool is rapidly lost when other nucleosides are added to the suspending medium. The half-time of efflux is less than 15 sec when a 200-fold excess of unlabeled adenosine, uridine or inosine is added to cells after the plateau level has been attained. A different pattern is obtained when free bases are added to the suspending medium (Fig. 3). The addition of adenine causes a slower depletion of the radioactive pool (half-time approx. 20 sec). Uracil, on the other hand, causes an initial rapid rise in the level of accumulation which is then followed by a comparatively slow loss of radioactivity. Other experiments have shown that methylated xanthines, including caffeine, have comparatively little effect on the level of accumulation. Radioactivity does not leak from cells at significant rates when cells are washed in 0° medium, suggesting that treatment with *N*-ethylmaleimide does not cause the formation of large pores in the membrane or appreciably block energy metabolism.

Because efflux is speeded by nucleosides and because of the very rapid rates of efflux, it also appears unlikely that phosphorylated compounds make the major contribution to the pool in the *N*-ethylmaleimide-treated cells since such compounds ordinarily penetrate the cell membrane very slowly. The additional observation that 2,4-dinitrophenol also causes a rapid loss of accumulated radioactivity down to 20% of the original activity (Fig. 2) indicates that the accumulation is energy dependent. Azide or 2,4-dinitrophenol blocks the initial entry of all nucleosides tested. Taken together it seems reasonable, therefore, to conclude that an active transport mechanism is reponsible for the accumulation against a concn. gradient of nucleoside by this strain of cells treated with *N*-ethylmaleimide, and probably for untreated cells as well.

Differential characteristics of nucleoside binding sites

Nucleoside transport sites for different purines and pyrimidines show marked differences in sensitivity to inactivation by heat and sulfhydryl-binding agents. Fig. 4 shows the rates of the three cellular reactions after cells have been heated for 15 min at various temperatures. Cellular adenosine deamination is most sensitive to this treatment. The rate falls markedly above 45° and is reduced to near 0 at 50°. On the other hand, cellular cytidine deamination is only slightly affected by exposure to the same temperatures. It should be emphasized that, although the enzymes present in these cells are inactivated to some extent at the elevated temperatures, sonicates of these treated cells show that the enzymes are present in an apparent 3-fold excess at the lower temperatures and that the excess increases to over 10-fold at temperatures near 50°. Thus, it is possible to destroy almost completely the cell's ability to transport adenosine and yet retain a significant portion of the cytidine and uridine transport mechanisms. This indicates that adenosine transport involves a thermolabile element not involved in cytidine or uridine transport.

p-Chloromercuribenzoate (PCMB) is also rather specific in its reaction with pyrimidine nucleoside transport sites. A 3-min exposure of cells to 0.1 mM PCMB decreases the rate of uridine cleavage to less than 2% of the untreated rate. Yet the same cells were still capable of deaminating cytidine at a relatively high rate, being inhibited only 42%. Since neither the internally located deaminase nor the cleavage enzyme were inactivated by the addition of PCMB, the results indicate that the uridine transport element inactivated by the mercurial does not function in the transport of cytidine.

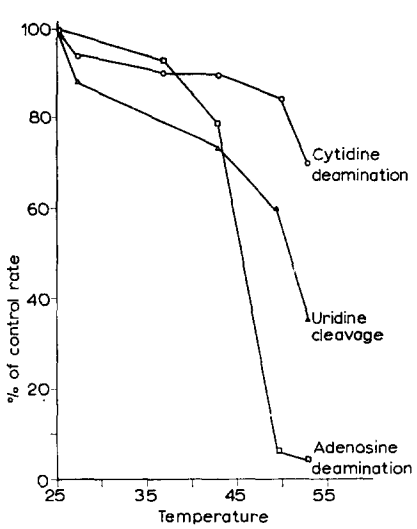


Fig. 4. Heat inactivation of adenosine and cytidine deamination and uridine cleavage by intact cells. Washed cells were heated directly in a water-bath at the indicated temperatures for 15 min and then cooled to room temperature. Cells were then washed and resuspended in fresh medium before rate determinations were made. Measurements are reported relative to the control rate where the cells were kept cold until a few minutes before assay. In all cases assays were conducted at 28°.

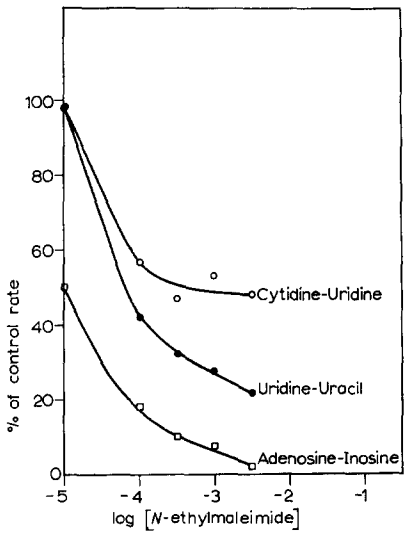


Fig. 5. *N*-Ethylmaleimide inhibition of the intact cell deamination and cleavage reactions at 28°. Washed cells were treated with freshly prepared *N*-ethylmaleimide at the indicated concentrations for 15 min at 28°. Cells were then centrifuged, washed and resuspended in fresh medium before rate determinations were made. The final concentration of each substrate was 100 μM.

Besides its effect on nucleic acid synthesis, *N*-ethylmaleimide also reacts with components of the nucleoside uptake mechanisms. This is shown in Fig. 5 where the rates of the 3 cellular reactions after treatment with increasing concentrations of *N*-ethylmaleimide are compared. Low concentrations of *N*-ethylmaleimide markedly depress the rate of adenosine deamination while much larger concentrations of the reagent are needed to cause a similar reduction in the rate of cytidine deamination. Interestingly, the rate of cytidine deamination can only be decreased to half the maximal values, even with excess *N*-ethylmaleimide; *i.e.* 50% inhibition is observed with *N*-ethylmaleimide concentrations ranging from 0.1 to 3 mM. This suggests that perhaps more than one kind of site mediates the transport of this nucleoside. We will return to this point below.

Cellular cytidine deamination is protected from inactivation by *N*-ethylmaleimide by itself and by other nucleosides if present in the reaction medium. This is similar to the finding of FOX AND KENNEDY[6] that certain galactosides can protect the galactoside permease system against *N*-ethylmaleimide. Table IV shows the results of experiments in which the degree of inactivation of the three cellular transports was measured after treating cells with 0.5 mM *N*-ethylmaleimide. Cellular cytidine deamination is most readily protected by each of the three nucleosides; in this case cytidine and uridine are the most effective protectors. Adenosine transport is significantly protected only by adenosine itself, and the inhibition of uridine

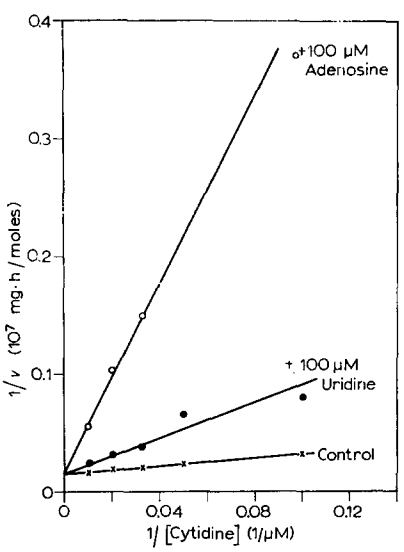
TABLE IV

ABILITY OF NUCLEOSIDES TO PROTECT TRANSPORT SITES FROM INACTIVATION BY *N*-ETHYLMALEIMIDE

Reaction with *N*-ethylmaleimide was carried out at 28° for 15 min in the presence of 50 μg/ml chloramphenicol. Cells were then washed and resuspended in fresh medium. Assays were conducted in the presence of substrate only. The per cent inhibition is based on the rate of transport of the non-*N*-ethylmaleimide-treated control.

Treatment	*% inhibition*		
	Cytidine deamination	*Adenosine deamination*	*Uridine cleavage*
N-ethylmaleimide (0.5 mM)	46.3	83.0	56.3
N-ethylmaleimide + 1 mM cytidine	6.9	82.9	50.3
N-ethylmaleimide + 1 mM adenosine	21.3	32.9	54.5
N-ethylmaleimide + 1 mM uridine	9.6	81.1	58.1

cleavage is not reduced by the presence of any of the nucleosides tested. Thus, while uridine is capable of protecting the cytidine mechanism, it does not effectively protect its own site nor that involved in adenosine uptake. This further suggests that distinct and separate elements function in the transport of the three nucleosides. The protection effects are increased as the protector concentrations are increased and are decreased at higher concentrations of *N*-ethylmaleimide.



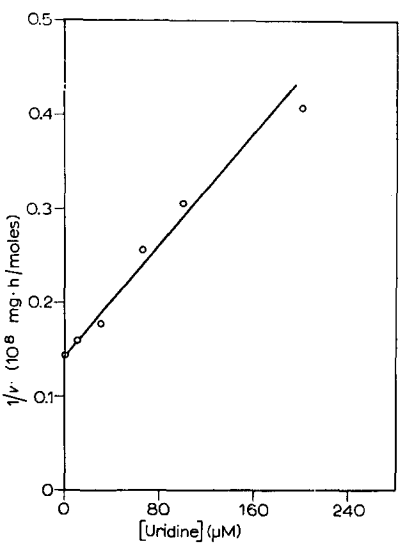


Fig. 6. Competitive inhibition of cellular cytidine deamination by adenosine and uridine. Rates were measured at 28° (uridine-adapted cells) in medium containing chloramphenicol (10 μg/ml). The K_i values, calculated from simple competitive theory, are: adenosine, 2.4 μM; uridine, 11.6 μM.

Fig. 7. Effect of increasing uridine concentration on the rate of cytidine deamination. Rates were determined at 28° for cells adapted to grow in uridine as sole carbon source. The cell suspension contained chloramphenicol (10 μg/ml), 25 μM cytidine and 1.0 × 10^8 cells. The K_i for uridine, calculated from simple competitive theory, is 11.7 μM.

Since both uridine and adenosine can protect the cytidine binding site, it might be expected that both compounds should competitively inhibit cytidine deamination in intact cells. This is indeed what is observed in double reciprocal plots made at constant inhibitor concentration (Fig. 6). Adenosine is a more effective inhibitor than uridine but it should be noted that the reverse was true for protection against *N*-ethylmaleimide inactivation.

When the reciprocal of the velocity of cellular cytidine deamination is plotted against the concentration of uridine as inhibitor, holding the substrate concentration constant, inhibition also appears to be nearly purely competitive (Fig. 7). Moreover, the same values of K_i (11.6 μM from Fig. 6 and 11.7 μM from Fig. 7) are obtained in the 2 types of experiment.

Usually, observation of competitive behavior is taken as evidence of a single site, in the present case with the assumption that adenosine and uridine interact directly with the same site that is responsible for the transfer of cytidine into the cell. However, from the findings listed above this site is not that used for the transport of adenosine or inosine. Additional evidence results from the observation that if cells are treated with PCMB, uridine cleavage is nearly abolished; but uridine is still a potent inhibitor of the residual 53% cytidine transport sites and at a concentration of 200 μM reduced the rate of cytidine deamination by 77%. This is just about the degree of inhibition to be expected from non-PCMB-treated cells. Adenosine is also an effective inhibitor under these conditions and caused a 92% reduction in the rate of cytidine deamination when added at a concentration of 200 μM.

Still more evidence that inhibition of transport of another nucleoside by 2 a nucleoside is due to a different interaction than its own transport results from experiments in which cells are heated at 51° for 15 min and then treated with 0.5 mM *N*-ethylmaleimide. This treatment inactivates the adenosine transport mechanism

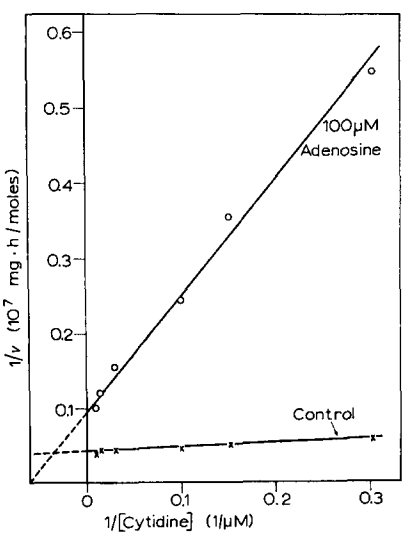


Fig. 8. Lineweaver–Burk plot of adenosine inhibition of cytidine deamination using cells pretreated by exposure both to heat for 15 min at 51° and 5 mM *N*-ethylmaleimide under standard conditions. Before rates were determined, treated cells (0.16 mg dry wt.) were washed and resuspended in fresh medium containing chloramphenicol (10 μg/ml).

almost completely but does not prevent adenosine from inhibiting residual cytidine transport sites. In this case, the inhibition is no longer purely competitive (Fig. 8). A number of experiments with untreated cells have been carried out, and the kinetic behavior was always purely competitive, like the results of Fig. 7. *N*-Ethylmaleimide together with heat treatment in a number of experiments resulted in a system where the maximum velocity was significantly lowered by the inhibitor, similar to the results of Fig. 8.

From these observations it would appear that the sites for interaction for adenosine and uridine which exert their effects on cytidine transport are not involved in the transport of the inhibiting nucleosides. Also indicative that separate binding sites are involved is the finding that the K_i for uridine inhibition (11.7 and 11.6 μM, calculated from Figs. 6 and 7) is almost four times the K_m for uridine uptake (3.3 μM). This difference is, however, not general; thus K_i for adenosine inhibition of cellular cytidine deamination is 2.4 μM (Fig. 6); but, as a substrate for transport, K_m is nearly the same (2.7 μM)[1].

Only marginal differences between those cytidine transport sites which show a resistance and those which show a sensitivity to *N*-ethylmaleimide have been found. When tested, the apparent K_m for uptake in *N*-ethylmaleimide-treated cells was approx. 6.0 μM; for untreated cells independent runs gave K_m values of 4.1 μM (Table I), 4.6 μM (Fig. 1) and 3.1 μM. Inosine and guanosine, which weakly interact with the cytidine uptake mechanism in untreated cells, show virtually no interaction with the sites that are insensitive to mercurials or *N*-ethylmaleimide. Furthermore, the resistant sites show the same response to azide as do the sensitive ones.

DISCUSSION

A scheme for nucleoside transported resembling the permease model[7,8] has been presented[1] in which separate transport elements were involved in the uptake of purine and pyrimidine nucleosides but were linked through a common element presumed to be the carrier[3,8–10]. The present report has presented evidence demonstrating further distinctions in the various nucleoside transport sites. The adenosine uptake site contains a more thermolabile component which can be inactivated without a similar reduction in the rate of uridine and cytidine uptake. Thiol reagents inactivate the uridine and adenosine sites but only partially inactivate the cytidine transport mechanisms.

Both adenosine and uridine, however, are capable of strong competitive interaction with the cytidine transport mechanism under conditions in which no significant uptake of the inhibiting nucleosides occurs. This is not an isolated instance; in other experiments we have found that a heat treatment of 51° for 15 min, which reduced cellular adenosine deamination to less than 1%, resulted in cells in which 50 μM adenosine inhibits uridine cleavage 35% when the uridine concentration is 10 μM and 24% when the uridine concentration is 100 μM.

Thus, it may be typically the case that transport for a particular nucleoside may still be inhibited by another nucleoside that could be transported across the membrane, but is not transported because certain elements involved in its specific transport have been inactivated.

A priori, it is not likely that a number of specific permease systems exist which

are all capable of binding a wide variety of nucleosides but are each capable of catalyzing the entry of only one nucleoside. Experimental objections to this are our present finding that all natural nucleosides tested speed uridine efflux and our previous finding[1] that inosine efflux is speeded by purine and pyrimidine nucleosides. Both findings suggest a common carrier for nucleosides. Further, the interactions resulting in competitive inhibition can not result from completely independent transport systems coupled only by an internal pool of accumulated nucleosides.

Ordinarily competitive inhibitors are such because, although they combine with the enzyme, they react to form product either slowly or not at all. Presumably, the action of a permease is simply to transfer the substrate intact to the carrier. If this is correct, it is difficult to see how a permease could bind a nucleoside and not be capable of transferring the bound nucleoside over to the less specific carrier element. While unusual, such specificity of the catalytic reaction, as distinct from the binding reaction, can not be considered unique since enzymes with this kind of specificity may be needed by replicases[11].

The type of inhibition by adenosine of cytidine deamination found in cells treated with *N*-ethylmaleimide and heat seems to be no longer simply competitive, but partially competitive or of even a more complicated type. Not only does the inhibitor cause the intercept with the ordinate of the Lineweaver–Burk plot to change, but it also changes the intercept with the abscissa. Several explanations for this kind of interaction must be considered. According to the current concepts of flexible enzymes and allosteric interaction, partial non-competitive inhibition may result from binding of the inhibitor to a site close to but different from the binding site. This binding results in a modification of the active site which might decrease its apparent affinity for substrate and/or cause an appreciable change in v_{max}. Such interaction could explain the ability of adenosine and uridine to protect the catalytic site from inactivation by *N*-ethylmaleimide since a change in the conformation of the active site could decrease the susceptibility of active sulfhydryl groups to the attacking reagent.

The suggestion of an allosteric mechanism to regulate the entry of exogenous nutrilites may have some justification from the point of view of advantageous metabolic control. In many biosynthetic pathways pronounced allosteric effects are often found at the initial step in the synthetic sequence. Since the initial step (and often the rate-limiting step[8]) in the utilization of exogenous substrates is permeation, this step might well be subject to some form of metabolic control. In the case of nucleoside transport such control mechanisms are probably not related to the utilization of these compounds as materials for macromolecular syntheses, since the rates of nucleoside entry can exceed, by as much as 50-fold, the cell's needs for purines or pyrimidines[2]. However, the aromatic amino groups of adenosine and cytidine are excellent sources of nitrogen for these cells. Earlier studies (R. N. PETERSON, unpublished) had shown that growth rates in the presence of either nucleoside as nitrogen source were comparable to those obtained in medium supplemented with ammonium chloride, even at growth-limiting concentrations of the nucleoside. Thus, regulation could be related to a necessity for controlling the influx of the utilizable nitrogen into the cell. The ability of these cells to utilize the ribose moiety of nucleosides as carbon sources may also provide justification for controlling entry rates.

If this turns out to be the case, it will be a novel type of control, since it represents a control of the action of an enzyme (*i.e.* permease) through the aegis of another exogenous substrate (*i.e.* a second nucleoside). There are, however, two possible objections to the allosteric hypothesis. Under such regulation the cell could not preferentially deaminate one nucleoside so that it would not appear to be a useful control. Secondly, all the kinetics we have obtained yield hyperbolic dependencies on substrate concentration; and there has been no evidence for a homotrophic interaction, as is frequently found for allosteric interactions.

An alternative explanation for the nucleoside competition and protection is that the interaction occurs through a common transport element. The competition results can be interpreted in this way since a single entity would bind all nucleosides. The protection of the transport of a nucleoside against sulfhydryl reagent inactivation by a second nucleoside without protection of the transport of the second nucleoside would be much more difficult to explain; it would require the assumption of new or more complicated elements than have so far been postulated in the transport process.

For example, suppose that uridine and adenosine slow cytidine entry by competing for a common carrier element after reaction with a primary and more specific element (*e.g.* permease). It would then be necessary to suppose that the mercurials and *N*-ethylmaleimide do not affect the binding of uridine and adenosine to the first element but rather inactivate an active center on the carrier element. Allosteric sites on the carrier might then account for further inhibition of cytidine transport. It is obvious, in any event, that pinpointing the exact nature of these interactions requires a further dissection of the nucleoside transport mechanisms. The final analysis must explain the interesting paradoxes that inactivation of the transport mechanism for a particular nucleoside does not prevent that compound from inhibiting the transport of another nucleoside, and that it may be able to protect the transport mechanism of another nucleoside without being able to protect its own site.

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