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REGULATION OF URACIL UPTAKE IN *ESCHERICHIA COLI* BY ADENOSINE 3′,5′-MONOPHOSPHATE

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SUMMARY

Culture of a wild-type strain of *Escherichia coli* in the presence of cyclic AMP leads to an impairment of uracil uptake. Half maximum inhibition of uracil uptake was observed at 1.5 mM cyclic AMP. The effect seems to be specific since no inhibition was found in cultures supplemented with ATP, ADP or 5′-AMP. Similarly the inhibition was not observed in cultures of a mutant deficient in the cyclic AMP receptor protein.

The inhibition in uracil uptake, found in bacteria cultured in the presence of cyclic AMP, is not a consequence of a reduction in the growth rate. On the other hand, this inhibition was observed only in cultures containing glucose or pyruvate as carbon source.

INTRODUCTION

Evidence obtained in this laboratory indicated that the presence of adenosine 3′,5′-monophosphate (cyclic AMP) in the culture medium decreases the rate of *Escherichia coli* cell multiplication. This inhibition seems to be specific for the cyclic nucleotide and was observed at concentrations similar to those required for reversal of permanent catabolite repression [1]. Cyclic AMP-promoted inhibition of growth varies with the carbon source present in the culture medium. This effect is observed in bacteria cultured in glucose or pyruvate but not in succinate, malate or glycerol. Some evidence was obtained indicating that the lack of effect is probably due to the relatively high levels of the nucleotide within the cells growing in the latter carbon sources [2].

According to these results, the restriction imposed by cyclic AMP on bacterial multiplication should be considered an expression of a more general phenomenon involving, for example, macromolecular synthesis (DNA, RNA, protein) or membrane transport of precursors, and not a consequence of an impairment of some enzymatic activity related to the metabolism of a given carbon source.

Evidence reported in this paper shows that the presence of cyclic AMP in *E. coli* cultures leads to an impairment in uracil incorporation into RNA. Such a

phenomenon appears to be a consequence of the inhibition produced at the level of precursor uptake.

A preliminary account of this work has been published elsewhere [3].

MATERIALS AND METHODS

Bacterial cultures

Bacteria were grown in AT minimal medium [4] which contains 14 g of K_2HPO_4, 6 g of KH_2PO_4, 2 g of $(NH_4)_2SO_4$ and 0.2 g of $MgSO_4$ per liter, supplemented with 5 μg/ml of thiamine. The different carbon sources, e.g. glucose, succinate, pyruvate or malate, were used at 25 mM. Overnight cultures were diluted with fresh medium to an absorbance at 550 nm of about 0.05. Aliquots of this bacterial suspension were cultured in a water-bath shaker at 37 °C with the additions detailed for each case.

Cell growth was followed either by measuring the increase in absorbance at 550 nm (1 cm light path) or by counting the number of cells by means of a Petroff-Hausser chamber. Similar results were obtained by the two methods [1]. The increase in absorbance and cell number was measured in cultures containing or not containing 5 mM cyclic AMP, and it was compared with the increase in cell mass (dry weight). Under both conditions the equivalence found was an absorbance unit equivalent to $3.5 \cdot 10^8$ cells/ml, equivalent to 0.40 mg/ml. These relationships are in reasonable agreement with those reported by other authors [5–7].

Strains

The following bacterial strains were used throughout this work: PP6 (wild-type, HfrH, thia$^-$, also known as Hfr 1100) and PP47, a nitrosoguanidine mutant of PP6, cyclic AMP receptor protein deficient (also known as Hfr 5333), kindly provided by Dr. I. Pastan.

Uptake assays

Determinations of [^{14}C]uracil or [^{3}H]uracil uptake or precursor incorporation into the trichloroacetic acid-insoluble fraction were performed on aliquots of exponentially growing cultures, adjusted to the same absorbance (between 0.1 and 0.3) with cold medium, using the rapid filtration technique [8, 9]. Bacterial samples were prewarmed and then incubated without shaking at 37 °C with the precursor concentration detailed in each case and for the indicated periods. The total volume was about 0.1 ml. Reactions were stopped by dilution with 4 ml of cold AT minimal medium and rapidly filtered through nitrocellulose filters (Selectron 0.45 μm pore) and washed twice with fresh medium. Dilution, filtration and washing took no more than 15 s. Precursor incorporation into trichloroacetic acid-insoluble material was determined by a similar procedure, except that a cold 10 % trichloroacetic acid solution was employed instead of the cold medium for stopping and washing. Filters were dried and counted for radioactivity using a toluene-Omniflour (New England Nuclear Corp.) mixture.

Some experiments, such as those shown in Figs. 2, 3 and 5 were carried out according to a procedure similar to that used by McCarthy and Britten [10]. Incubations were performed in 10 ml Erlenmeyer flasks containing 1 ml aliquots of each

culture, at 37 °C with shaking. They were started by the addition of 0.05 ml of a solution containing [^{14}C]uracil. At the indicated times 0.05 ml aliquots were taken, diluted and processed as above described.

Uptake into the trichloroacetic acid-soluble fraction was calculated as the difference between values corresponding to the total uptake and those corresponding to the incorporation into the trichloroacetic acid-insoluble fraction. Precursor uptake or incorporation into trichloroacetic acid-insoluble material was expressed as nmol per 10^8 cells.

Blanks for precursor uptake or incorporation performed with non-incubated samples or with samples without cells were below 1 % of the total radioactivity in the corresponding aliquots.

Assay for β-galactosidase synthesis

The rate of enzyme synthesis was determined on aliquots removed from logarithmic cultures at different times after induction with 0.5 mM isopropyl β-D-thiogalactoside. Enzyme activity was assayed on toluenized cells according to the method of Wallenfels [11]. β-Galactosidase rate of synthesis was expressed as enzyme units/10^8 cells per min of induction.

Reagents

2-[^{14}C]Uracil, 5,6-[^{3}H]uracil and [u-^{14}C]leucine were purchased from New England Nuclear Corp. In some samples of radioactive uracil differences were observed between the indicated concentration and those determined spectrophotometrically; the latter values were considered more reliable. On the other hand, chromatographic purity of these samples checked by the procedure of Wyatt [12] varies with the time of storage at −20 °C. These facts could explain some differences found between specific activities previously reported [3].

Unlabelled uridine, uracil, isopropyl β-D-thiogalactoside and *O*-nitrophenyl-β-galactopyranoside were purchased from Sigma.

RESULTS

Effect of cyclic AMP on [^{3}H]uracil and [^{14}C]leucine incorporation

In order to determine the reason for the inhibition of bacterial multiplication by cyclic AMP, the effect of this compound on RNA and protein synthesis was studied.

As it is shown in Fig. 1, the rate of [^{3}H]uracil incorporation tended to decrease during the early exponential phase of growth. The phenomenon was much more evident in cultures carried out in the presence of cyclic AMP (generation time 98 min) than in those performed in the absence of the cyclic nucleotide (generation time 75 min). At the level of [^{14}C]leucine incorporation similar changes were observed. However, in this case the effect of cyclic AMP was less marked.

It is important the emphasize that the inhibition of uracil incorporation did not appear to be an immediate consequence of the presence of cyclic AMP in the culture. This effect was evident only after growing the bacteria in the presence of the cyclic nucleotide for about one generation time, and it tended to diminish after a period of about two additional generation times. The explanation might be that

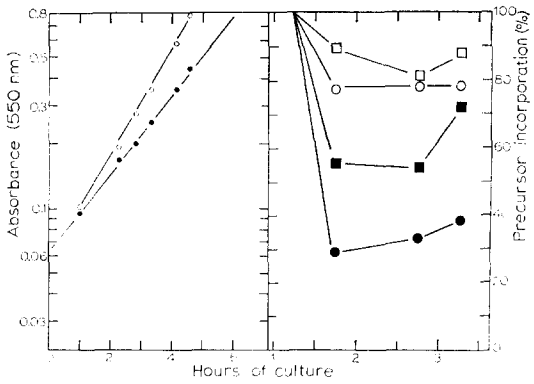

Fig. 1. Effect of cyclic AMP on cell growth rate and precursor incorporation. (A) Cultures were grown in media containing (●) or not containing (○) 5 mM cyclic AMP. (B) At the indicated times, aliquots from these cultures were taken and assayed for incorporation of precursors into the trichloroacetic acid-insoluble fraction. The assays containing 1.4 μM [^{3}H]uracil (specific activity 1.25 μCi/nmol) or 40 μM [^{14}C]leucine (specific activity 1.25 μCi/nmol) were performed for 2, 4, 6 and 10 min. From the values thus obtained, precursor incorporation rates were calculated and expressed as percent of those found in the aliquots taken at the time 75 min. Zero time corresponds to the moment in which cultures were started. For [^{3}H]uracil incorporation, the values were 0.040 and 0.029 nmol/min per 10^8 cells in aliquots from cultures containing (●) or not containing (○) cyclic AMP respectively; for [^{14}C]leucine the corresponding values were 0.114 and 0.125 nmol/min per 10^8 cells, respectively, for the aliquots taken from cultures containing (■) or not containing (□) cyclic AMP. Other conditions were as indicated under Materials and Methods.

the cyclic nucleotide would influence precursor incorporation only in the early exponential phase of growth.

Kinetics of uracil uptake and incorporation into the trichloroacetic acid-insoluble fraction in cultures supplemented or not with cyclic AMP

Total uracil uptake and its incorporation into the trichloroacetic acid-insoluble fraction in cultures growing exponentially either in the presence or in the absence of cyclic AMP followed kinetics similar to those found by Britten and coworkers [10, 13]. At uracil concentrations of about 1.7 μM the following facts characterized this kinetics. (1) Total uracil uptake was linear with time until more than 30 % of the nucleobase was exhausted from the medium. Thereafter the rate decreased (Fig. 2A). (2) In cultures performed in the presence of 5 mM cyclic AMP a reduction in the rate of uracil uptake of between 30 and 70 % of the value measured in unsupplemented cultures was observed (Fig. 2A). (3) Uracil incorporation into the trichloroacetic acid-insoluble fraction showed two phases. There was an initial incorporation, linear with the incubation time, and then, when more than 30 % of the nucleobase was exhausted from the medium, the rate declined (Fig. 2B). (4) During the linear initial phase of incorporation into the acid insoluble fraction, the rate was 55–65 % of that corresponding to the total uracil uptake (compare Figs. 2A and 2B). This fact was evident even under conditions in which the acid-soluble fraction was almost saturated with radioactivity (Fig. 2C). This result, similar to that obtained by McCarthy and Britten [10], did not agree with the interpretation of Nierlich, stated under the premise that "the rate of entry of uracil or guanine into cells did not exceed the rate

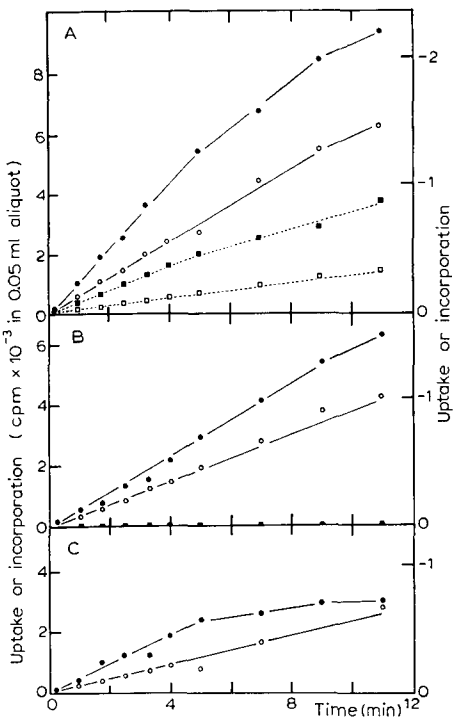

Fig. 2. Effect of cyclic AMP and rifampicin on total uracil uptake and incorporation into the trichloroacetic acid-insoluble fraction. Cultures were grown in media containing (○, □) or not containing (●, ■) 5 mM cyclic AMP and adjusted to an absorbance of 0.135. Aliquots from these cultures (1ml) were preincubated (■, □) or not (●, ○) for 15 min at 37 °C in the presence of rifampicin (0.30 mg/ml). After that, incubations for precursor uptake or incorporation were initiated by the addition of 0.05 ml of a solution containing 36.6 μM [^{14}C]uracil (0.08 μCi/nmol). At the indicated times, 0.05 ml aliquots were removed for determination of total uracil uptake (A), or incorporation into the trichloroacetic acid-insoluble fraction (B). Incorporations into the trichloroacetic acid-soluble fraction (C) were calculated from values given in A and B. Total radioactivity in a 0.05 ml aliquot of each incubation mixture was 15,700 cpm; reaction blanks were about 80 cpm. Other conditions were as described under Materials and Methods.

of their utilization in the synthesis of RNA" [5]. In addition, during the linear initial phase, the rate of precursor incorporation in cultures not supplemented with the cyclic nucleotide was similar to that reported by Bremer and Yuan [7]. More than 90 % of the material labeled during this phase seemed to be RNA according to its sensitivity to alkaline hydrolysis. (5) In cultures performed in the presence of cyclic AMP, the reduction of the rate of uracil incorporation into the acid-insoluble material paralleled that found at the level of total uracil uptake (compare Figs. 2A and 2B). It is important to note the striking proportionality found between the rate of precursor incorporation into the acid-insoluble material and those corresponding to the total uracil uptake or to the incorporation into the acid-soluble cell fraction (Fig. 3). A decrease in the latter rates, obtained by culturing cells in the presence of cyclic AMP, was coincident with a corresponding diminution of the rate of labelling of the acid-insoluble fraction. (6) Uracil uptake by *E. coli* cells resulted in a saturable process

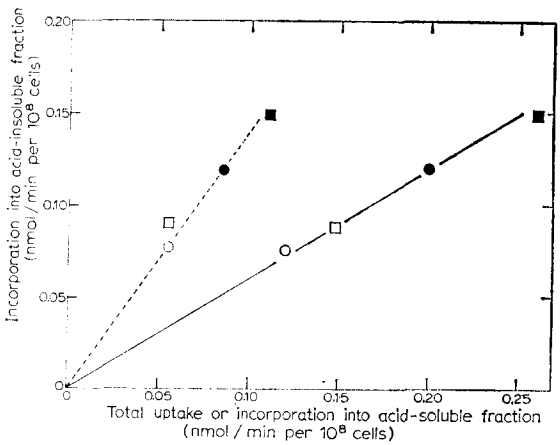

Fig. 3. Correlation between uracil incorporation into the acid-insoluble fraction and total uptake or incorporation into the acid-soluble cell fraction. Incorporations into the acid-insoluble cell fraction were plotted as a function of total uracil uptake (full lines) or incorporation into the acid-soluble cell fraction (broken lines). Squares and circles indicate values corresponding to two different experiments, one of them shown in Fig. 2. Closed symbols corresponded to cultures performed in the absence of cyclic AMP; open symbols corresponded to cultures containing 5 mM cyclic AMP.

showing only one kinetic component. Culture of bacteria in the presence of cyclic AMP leads to a decrease in the maximum velocity of this uptake. The apparent dissociation constant for the nucleobase was 0.6 μM (Fig. 4).

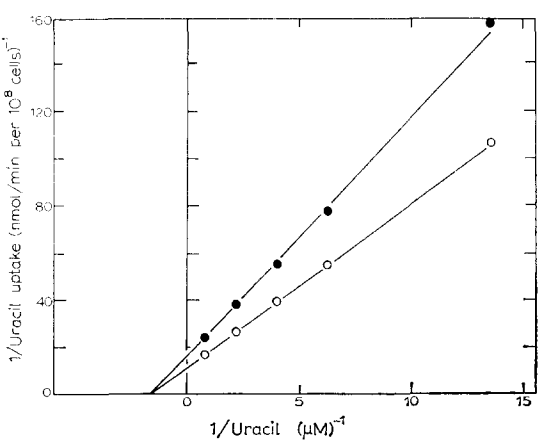

Fig. 4. Effect of cyclic AMP on kinetic parameters of total uracil uptake by *E. coli* cells. Bacteria were grown in the absence (○) or in the presence (●) of 5 mM cyclic AMP. Aliquots from these cultures were assayed for total uracil uptake at different concentrations of [^{3}H]uracil (0.074–1.33 μM; specific activity 2–100 μCi/nmol). Reciprocal initial rates of uracil uptake were plotted as a function of reciprocal concentrations of the base.

Kinetics of uracil uptake in cultures supplemented or not with cyclic AMP and treated with rifampicin

The possibility that transport processes might play a role in the regulation of total uracil uptake by *E. coli* cells could be neglected according to previous statements [5, 14]. Therefore, evidence indicating that uracil incorporation was affected by culturing cells in the presence of cyclic AMP could be taken as an indication of some inhibitory effect at the level of RNA synthesis. The incorrectness of this assumption was evidenced in the following results. (1) Uracil entered the cells under conditions in which precursor incorporation into the acid-insoluble fraction was undetectable (Fig. 2A and 2B). Such conditions were attained after cell preincubation for 15–20 min at 37 °C in the presence of 0.3 mg rifampicin/μl. (2) The initial rate of total uracil uptake in rifampicin-treated cultures (not supplemented with cyclic AMP) was 86 % of the corresponding rate of incorporation into the acid-soluble fraction of the control culture, not treated with the antibiotic (compare Figs. 2A and 2C). In different experiments this percentage varied between 50 and 86 %. (3) Inhibition of precursor uptake by cyclic AMP was also observed in the rifampicin-treated culture (Fig. 2A).

According to this evidence, the statement that "uptake of uracil would cease where there was no net synthesis of RNA" [5] cannot be longer accepted.

Characterization of cyclic AMP action on uracil uptake

As was shown in Fig. 1, inhibition of uracil uptake required the culture of bacteria in the presence of the cyclic nucleotide. This was also supported by the fact that addition of cyclic AMP at concentrations of about 5 mM to the assay mixtures did not modify the rate of precursor uptake by cells cultured in the absence of the cyclic adenylate (Fig. 5A). This result confirmed a similar observation of de Crom-

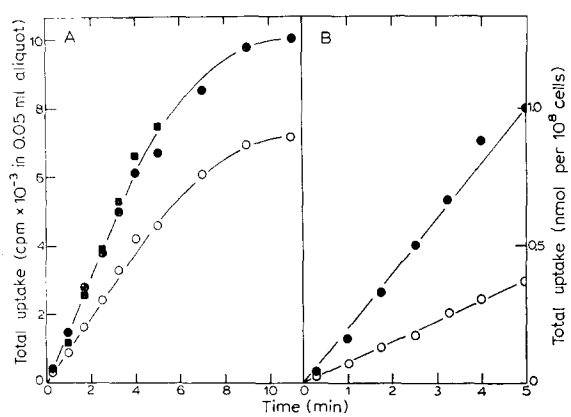

Fig. 5. Characterization of cyclic AMP action on total uracil uptake. Cultures were done in the presence (○) or absence (●, ■) of 5 mM cyclic AMP and adjusted to an absorbance of 0.23. (A) Aliquots from these cultures (1 ml) were incubated for precursor uptake. In addition, an aliquot from the culture performed in the absence of the cyclic nucleotide was assayed for total uracil uptake immediately after the addition of cyclic AMP to a final concentration of 5 mM. (B) Aliquots from each culture were centrifuged and the supernatants discarded. The cell precipitates were then resuspended in the same original volume with fresh medium. Ordinate units, cpm per 0.05 ml aliquot or nmol per 10^8 cells were common to A and B. Other conditions were as described in Fig. 2.

brugghe et al. [15] showing that under conditions for the reversal of catabolite repression the addition of cyclic AMP did not affect precursor incorporation measured immediately after this addition. Such evidence provided further support for the assumption that the inhibition is not merely a competition between uracil and cyclic AMP for their entry into the cells. Results not shown in this paper indicated the existence of such competition for thymidine uptake.

On this basis it could be accepted that, as a consequence of growing bacteria in the presence of cyclic AMP, some modifications were produced at the level of the systems responsible for uracil uptake. As shown in Fig. 5B, these modifications were maintained after elimination of cyclic AMP from the culture medium, immediately before the assay.

Specificity of the cyclic AMP effect

Most of the work on uracil uptake was carried out in bacteria grown in the presence of 5 mM cyclic AMP. The inhibition observed could be analyzed in terms of a toxic nonspecific effect elicited by this high nucleotide concentration. In fact, maximal inhibition and half maximal effect were attained at 5 mM and 1.5 mM cyclic AMP, respectively (Fig. 6). However, cyclic nucleotide levels required for this effect are within the range of those necessary to relieve permanent catabolite repression of β-galactosidase synthesis [15] and to inhibit bacterial growth [1, 2]. In addition, the inhibition of uracil uptake resulted in a specific phenomenon in terms of the dependence on cyclic AMP. In fact, no inhibition was found in cultures of the wild-type strain Hfr 1100 grown in the presence of ATP, ADP or 5'AMP.

The influence of growing bacteria in the presence of cyclic AMP was also studied in a mutant strain deficient in the cyclic AMP receptor protein. As shown in Table I, the inhibitory effect of the cyclic nucleotide was not observed in cultures of this strain. This could simply indicate that this protein was required for the transcrip-

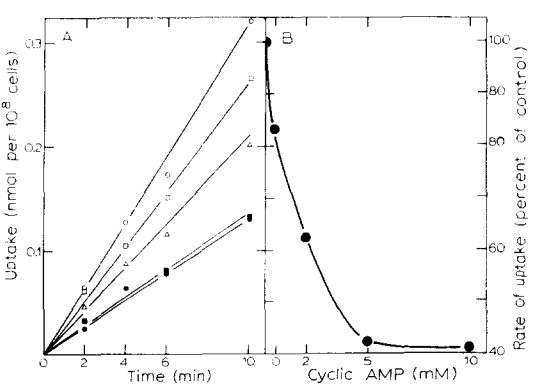

Fig. 6. Rates of uracil uptake as a function of cyclic AMP concentration in the growth medium. (A) Time course of total uracil uptake. Aliquots were taken from cultures grown in the presence of the following cyclic AMP concentrations: none (○); 0.5 mM (□); 2 mM (△); 5 mM (■) and 10 mM (●). (B) Rates of total uracil uptake measured in cultures containing cyclic AMP were expressed as percent values of that corresponding to the culture performed in absence of the cyclic nucleotide, and were plotted as a function of the concentration of this compound. Other conditions were as indicated in Fig. 1 and under Materials and Methods.

TABLE I

EFFECT OF NUCLEOTIDES ON THE RATE OF URACIL UPTAKE OF WILD-TYPE (Hfr 1100) AND CYCLIC AMP RECEPTOR PROTEIN DEFICIENT (Hfr 5333) STRAINS OF *E. COLI*

Growth medium of the latter strain contained 0.1 % casamino acids. Nucleotide concentration in the cultures was 5 mM. Other conditions were as indicated in Fig. 1 and under Materials and Methods

Strains	Nucleotide in the growth medium	Rate of total uracil uptake (nmol/min per 10^8 cells)
Hfr 1100 (PP6)	—	0.085
	ATP	0.088
	ADP	0.087
	5'-AMP	0.100
	Cyclic AMP	0.053
Hfr 5333 (PP47)	—	0.050
	Cyclic AMP	0.056

tion of some factor controlling cell permeability. However, recent evidence indicates that strains deficient in cyclic AMP receptor protein have abnormally high intracellular cyclic AMP levels [17]. Therefore, the absence of an effect of added cyclic AMP on these strains might be a reflection of the already high concentration of the cyclic nucleotide [18].

Correlation between uracil uptake and cell growth rate

Inhibition of uracil uptake by cyclic AMP was observed under conditions leading to a simultaneous impairment of cell growth. An important problem to solve was to determine whether the inhibition of uracil uptake was a direct consequence of growth retardation.

TABLE II

CORRELATION BETWEEN URACIL UPTAKE AND CELL GROWTH RATE

Cultures were grown at 37 °C and 30 °C for 3.5 and 4.5 h, respectively, in the presence or absence of 5 mM cyclic AMP. Thereafter, aliquots of each culture were immediately assayed for uracil uptake at 37 °C. In addition, other aliquots were treated with rifampicin (100 μg/ml) for 10 min at 37 °C and then assayed for uracil uptake at 37 °C. Other conditions were as indicated in Fig. 1 and under Materials and Methods.

Growth temperature (°C)	Doubling times (min)		Rate of uracil uptake (nmol/min per 10^8 cells)			
	No cyclic AMP	5 mM cyclic AMP	Before preincubation with rifampicin		After preincubation with rifampicin	
			No cyclic AMP	Plus cyclic AMP	No cyclic AMP	Plus cyclic AMP
37	57	85	0.080	0.056	0.056	0.036
30	87	115	0.088	0.058	0.052	0.028

A simple approach to solving this problem could be to study the correlation between uracil transport and growth rate in cultures carried out at different temperatures. Table II shows the results of an experiment performed with such approach. Assays for uracil uptake were carried out on bacterial samples previously treated or not with rifampicin. In the former case, further growth of bacterial samples at another temperature was avoided.

As can be seen, there was no correlation between the rates of cell growth and uracil uptake when the growth temperature was varied. That is, a decrease in the growth temperature from 37 to 30 °C led to a 50 % increase of the generation time found in the absence of cyclic AMP (57 to 85 min), and of a 32 % increase of those measured in the presence of this compound (87 to 115 min). Under these conditions the change in growth temperature did not influence uracil uptake.

These results suggest that the rate of cell multiplication did not influence per se the corresponding rate of precursor uptake.

Influence of different carbon sources

Our previous work on the control of bacterial growth has shown that the the lack of effect of cyclic nucleotide on bacteria growing in succinate or malate as carbon sources was a consequence of the intracellular accumulation of cyclic AMP elicited by these compounds [1, 2].

If the intracellular cyclic AMP level is one of the factors affecting uracil uptake, it could be expected that in cultures containing those carbon sources leading to catabolite repression (i.e. glucose or pyruvate) the presence of cyclic AMP might produce a decrease of uracil uptake. As shown in Table III, this seemed to be the case. The effect of cyclic AMP on uracil uptake was observed in cultures containing glucose or pyruvate but not in cultures containing malate or succinate. Similar dependence on the carbon source was observed at the level of cell growth rate, which was inhibited by the cyclic nucleotide, or at the level of β-galactosidase synthesis the rate of which was stimulated by this compound.

Table III also shows that there was no correlation between the rate of cell multiplication and uracil uptake, when cultures in different carbon sources were compared.

TABLE III

INFLUENCE OF CARBON SOURCES AND CYCLIC AMP ON THE RATE OF URACIL UPTAKE

Conditions were as indicated in Fig. 1 and under Materials and Methods.

Carbon	Doubling time (min)		Uracil uptake (nmol/min per 10^8 cells)		Rate of β-galactoside synthesis (units/min per 10^8 cells)	
	No cyclic AMP	5 mM cyclic AMP	No cyclic AMP	5 mM cyclic AMP	No cyclic AMP	5 mM cyclic AMP
Glucose	65	92	0.084	0.057	0.76	1.69
Malate	168	162	0.061	0.061	1.87	2.11
Succinate	166	170	0.077	0.085	1.83	1.88
Pyruvate	135	213	0.110	0.079	—	

DISCUSSION

Kinetics of uracil uptake

Under all conditions explored, the rate of radioactive uracil uptake was higher than the entry of the nucleobase into the acid-insoluble fraction. This was much more evident under the most extreme condition explored, that is in the presence of rifampicin, leading to a total inhibition of RNA synthesis.

Previous evidence, obtained from experiments in which pulse labelling of acid-insoluble material was chased by increasing concentrations of unlabelled exogenous nucleobase, indicated that the size of nucleotide pools was not affected by exogenous uracil [5, 10, 13]. However, experiments in the presence of rifampicin, such as those shown in Fig. 2A, indicated that at initial uracil concentrations of about 1.75 μM uptake by cells ceased after 12–15 min of incubation. Under these conditions, external precursor concentration fell to 1.25 μM and internal labelled products reached a maximal concentration of about 5 mM*. This value was high enough to suggest a contribution of pool expansion to the factors conditioning total uracil uptake.

The existence of an exchange reaction between pool and exogenous uracil was firstly indicated by Koch [19]. Experiments not shown here indicate that the ceasation of uracil uptake after 12–15 min of incubation with the labelled precursor was not the consequence of a complete inactivation of the transport system in the rifampicin-treated cells. Then it could be accepted that after this period the system reached a steady state. From the ratio of external uracil concentration (1.25 μM) to that of internal labelled products (5 mM), it could be inferred that the corresponding ratio for entry and overall exit rate constants should be about 4000.

Another striking property shown was that uracil incorporation into the acid-insoluble material proceeded linearly from the beginning of the incubation, without any detectable lag in the incorporation (see Fig. 2B). In addition, the rate of precursor incorporation into this material was, paradoxically, slightly higher than that into the soluble pool (compare Figs. 2B and 2C). These facts suggest that a very fast isotopic equilibration between the soluble pool and some fraction of the acid-insoluble material might occur. This rapid isotopic equilibration and the exchange with exogenous uracil were precisely pointed out by Koch [18] as the more important reasons that explain deviations of bacterial systems from the behaviour predicted by the "net synthesis theorem" [5, 20].

Influence of cyclic AMP on uracil uptake

This paper reports evidence indicating that the culture of *E. coli* in the presence of cyclic AMP leads to an impairment of uracil incorporation into the macromolecular acid-insoluble fraction. The influence of cyclic AMP on this incorporation seemed to be a direct consequence of the cyclic nucleotide effect at the level of uracil uptake.

Precursor incorporation into the trichloroacetic acid-insoluble fraction has been frequently taken as an indication of the rate of macromolecular synthesis (DNA,

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* This value was based on the following parameters: free minimal intracellular water, $0.75 \cdot 10^{-15}$ l/cell [6]; number of cells in 0.05 ml aliquot (absorbance 0.20), $0.035 \cdot 10^{8}$; total uptake, 14.7 pmol of radioactive uracil.

RNA, protein, etc.) in a variety of cellular physiological states. The results above discussed indicate that under certain conditions the rate of uracil incorporation into the macromolecular acid-insoluble material might be a function of the total uptake or of uptake into the acid-soluble fraction (see Fig. 3).

The molecular basis of the cyclic AMP-dependent control of uracil uptake by *E. coli* cells is not well understood. Several alternative explanations of the phenomenon could be advanced. (1) The first step leading to the conversion of uracil into the corresponding nucleoside phosphates might be subjected to a negative control, like feedback, by high intracellular cyclic AMP levels. (2) Under conditions of reversal of permanent catabolite repression by cyclic AMP, several changes of intracellular metabolite levels could be expected. In turn, these changes might influence reaction rates leading to the conversion of uracil to nucleoside phosphates through the feedback control mechanisms known to affect these conversions. (3) The cyclic nucleotide might promote, at the transcriptional level, the synthesis of a protein required for the control of membrane transport and/or the conversion of the nucleobase to nucleoside phosphates. (4) The cyclic nucleotide might induce some modifications in membrane components, leading to changes of cell permeability. The latter mechanism, the existence of which in eucaryotic cells has been evidenced [21, 22] should require bacterial cyclic AMP receptor protein(s) to play other roles in addition to the well known involvement in transcription of inducible operons [23, 24].

Cyclic AMP and the control of cell growth and precursor uptake

The control of cell growth in *E. coli* cells was strikingly analogous to that observed in eucaryotic cell line cultures (see ref. 24 for references). In the two systems, metabolite uptake also seemed to be under the control of factors affecting intracellular cyclic AMP levels.

The relationship between cell multiplication and metabolite transport has been remarked by some authors [26–28] suggesting that, under certain conditions, precursor uptake could be a limiting step for cell growth. However, it is important to emphasize that growth inhibition of a wild-type strain cultured in minimal medium cannot be explained in terms of some impairment at the level of uracil uptake.

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