CONTROL OF PYRIMIDINE BIOSYNTHESIS IN ESCHERICHIA COLI BY A FEED-BACK MECHANISM*

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(Received for publication, November 21, 1955)

The mechanisms by which a cell can control its biosynthetic processes are important from the point of cellular economy, yet they are little understood. It is known that bacteria must have such control systems, since their biochemical processes are so well coordinated, as shown by failure of the cell to accumulate large amounts of metabolites in the medium (1).

Several indications of control mechanisms have been found. One type of observation is that addition of a few micrograms per ml. of normal purines (2), pyrimidines (1), or amino acids (3, 1) to growth media of normal bacteria could result in nearly exclusive incorporation of these materials into cell constituents, in preference to *de novo* synthesis of the materials by the cell. It has been noticed also that production of intermediates of the above materials can be inhibited by addition of the end-products to cultures of suitable bacterial mutants. Gots and Chu (4) and Love and Gots (5) found that addition of purines to the culture medium of purine-requiring *Escherichia coli* mutants caused inhibition of formation of two purine precursors, and Brooke *et al.* (6) showed the inhibition of orotic acid (OA)[1] formation in the presence of uracil by pyrimidine-requiring *Aerobacter aerogenes* mutants. Adelberg and Umbarger (7) observed that the presence of the growth factor, valine, inhibited formation in an *E. coli* mutant of α-ketoisovalerate, a valine precursor, and commented that the unknown mechanism was undoubtedly wide-spread and important to the economy of the cell.

Work from this Laboratory indicated that the presence of uracil inhibited formation of pyrimidine intermediates earlier than orotic acid in pyrimidine-requiring *E. coli* mutants (8). It seemed of interest to determine whether this observation plus the above facts could be the results of normal self-regulatory mechanisms of the cell which allowed formation of no more

* Presented in part at the Pacific Slope Biochemical Conference, 121st meeting of the American Association for the Advancement of Science, December 30, 1954.

Supported, in part, by research grants from the Lederle Laboratories Division, American Cyanamid Company, and the Rockefeller Foundation.

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[1] The abbreviations used are as follows: OA = orotic acid (4-carboxyuracil); DHO = dihydroorotic acid; US = ureidosuccinic acid (carbamylaspartic acid); RNA = ribonucleic acid; DNA = deoxyribonucleic acid.

components than necessary for growth. For this reason studies *in vivo* and *in vitro* on the control of pyrimidine biosynthesis in *E. coli*, strain B, and two pyrimidine-requiring mutants of *E. coli* were carried out.

Materials and Methods

Uracil, cytosine, uridine, orotic acid, cytidine-5′-phosphate, and 4-C^{14}-DL-aspartic acid were obtained commercially. Cytidine was kindly provided by Dr. C. A. Knight and uridine-5′-phosphate by Dr. C. Dekker of these laboratories. DL-US and L-DHO were prepared as described in the accompanying paper (8). *E. coli* strain B and the two pyrimidine-requiring *E. coli* mutants (6386 and 550-460) were obtained and cultured in the minimal medium (containing salts and glycerol) described earlier, with uracil or OA as growth factor when needed (8). For inactivation of nucleic acid formation in the bacteria, freshly grown cells were washed, suspended in minimal salt solution at a concentration of 2.5×10^{10} cells per ml., and irradiated for 3 minutes in a large Petri dish (suspension 0.5 cm. deep) 14 inches below three Westinghouse sterile lamps No. WL-782L-30. Under the conditions used, both DNA and RNA syntheses by the cells were essentially abolished and fewer than 0.001 per cent of the cells were capable of colony formations on broth plates.

EXPERIMENTAL

Control in Vivo of Pyrimidine Intermediate Production by Mutants—In the study of pyrimidine-requiring mutant 550-460 it was noticed that large amounts of the pyrimidine intermediates US, DHO, and OA were produced only in media free of the pyrimidines uracil, cytosine, or their nucleosides or nucleotides (8). Under similar conditions mutant 6386 produced US and DHO only in the absence of the above pyrimidines. Thymine neither stimulated growth nor inhibited production of the intermediates. The effect of traces of uracil in this inhibition can be seen in Fig. 1, where production of the intermediates became significant only when the added uracil had been exhausted by cell growth. The above results extend the report by Brooke *et al.* (6) that OA formation was inhibited by the presence of uracil in pyrimidine-requiring *A. aerogenes* mutants.

It was necessary to determine whether the inhibition was a direct effect of the pyrimidine or was dependent in some way on cell multiplication, since under the above conditions cells were growing whenever the pyrimidine growth factor was added to the cultures. A substrain of *E. coli* mutant 6386, characterized by rapid growth with OA as a growth factor, was isolated. As shown in Fig. 2, the rate of growth was comparable in minimal medium containing OA or uracil, yet the rate of DHO production

per cell in OA-containing medium was even higher than production by cells suspended in minimal medium. This result indicated that the inhibition was specifically brought about by uracil. Apparently the uptake and conversion of OA to uridylic acid (9, 10) were rate-limiting for growth.

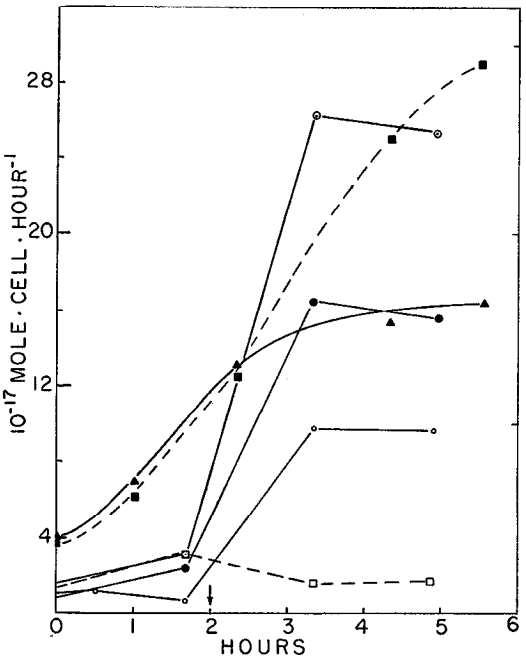

FIG. 1. Rate of production of US and DHO by *E. coli* mutant 6386 and dependence on concentration of uracil in the medium. See the text for details. ■ and ▲, cell count of culture containing initial concentrations, respectively, of 2.0×10^{-4} M and 5.0×10^{-5} M uracil. □, total rate of US plus DHO production in culture containing 2.0×10^{-4} M uracil. ○, ●, and ⊙, rates of production of DHO, US, and of DHO plus US, respectively, in culture containing 5.0×10^{-5} M uracil. Initial concentration of 1.0×10^{-4} M uracil allows formation of 1.5×10^{9} cells. The arrow indicates when the 5.0×10^{-5} M uracil should be exhausted.

Thus the inhibitory compound was used for nucleic acid synthesis as rapidly as it was formed from orotic acid and did not accumulate inside the cell.

If the above ideas are correct, inhibition of nucleic acid biosynthesis at a stage beyond the formation of nucleotides should result in inhibition of formation of the pyrimidine intermediates. To test this, washed mutant 6386 cells, freshly grown in minimal medium with OA as growth factor, were irradiated heavily with ultraviolet light to halt both DNA and RNA biosynthesis. These cells were then incubated in minimal medium and in

medium plus OA. In minimal medium the rate of DHO production per irradiated cell decreased slowly with time, presumably partly as a result of enzyme destruction (Fig. 3). Irradiated cells in medium containing OA, however, very soon showed a decrease in the rate of DHO forma-

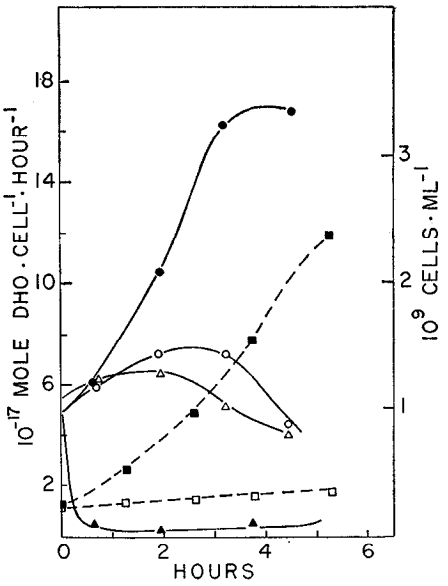

FIG. 2. Rates of pyrimidine intermediate production by growing and by non-growing mutant 6386 cells. Cultures of *E. coli* mutant 6386 cells grown on minimal medium plus OA or plus uracil were harvested and incubated for 1 hour in minimal media. Reharvested and washed, the cells were suspended in minimal medium alone or in media containing the same growth factor used before. □ and ■, cell count (turbidity) of cells suspended, respectively, in minimal media and in media containing 2×10^{-4} M uracil or orotic acid (growth rates nearly identical). △ and ▲, per cell rate of production of DHO by cells previously grown in media containing uracil and resuspended in minimal medium and in medium containing 2×10^{-4} M uracil, respectively. ○ and ●, per cell rate of production of DHO by cells previously grown in media containing OA and resuspended in minimal medium and in medium containing 2×10^{-4} M OA, respectively.

tion, the decrease being almost as rapid as that caused by the presence of uracil in the medium. The rate of inhibition by growth factor appears to be correlated with normal growth rates, since cells grew slightly faster with uracil than with OA. The results support the idea that when a cell does not use a uracil derivative as rapidly as it is produced from OA, this derivative accumulates and inhibits the formation of the pyrimidine intermediates US and DHO.

Estimation in Vivo of Inhibition Constant—The concentration of uracil

derivative in the cell necessary to cause half inhibition of the rate of DHO production was determined. The ability of ultraviolet-irradiated 6386 cells to remove uracil from the medium was measured by bioassay with mutant 550-460 (which requires uracil for growth). About 6.7×10^{-17}

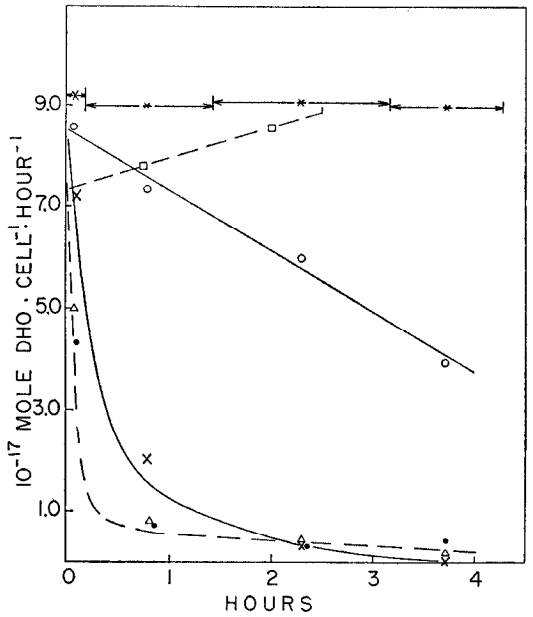

FIG. 3. Pyrimidine intermediate production by ultraviolet-irradiated mutant 6386. The cells were grown at 37° in minimal medium containing 2.0×10^{-4} M OA (division time 2.0 hours) and samples taken at intervals were assayed for DHO. The culture was centrifuged, washed, and suspended in minimal salts, then irradiated with ultraviolet light for 2 minutes and suspended at about 6.0×10^{8} cells per ml. of test medium. Samples were removed at intervals and tested for DHO. The average amount of DHO produced per hour per bacterium over the time intervals shown at the top of the graph is represented by the area under a plotted line. □, growing cells on minimal medium plus 2.0×10^{-4} M OA. ○, ultraviolet-treated cells on minimal medium. ×, ultraviolet-treated cells on minimal medium plus 2.0×10^{-4} M OA. △, ultraviolet-treated cells on minimal medium plus 1.0×10^{-5} M uracil. ●, ultraviolet-treated cells on minimal medium plus 1.0×10^{-4} M uracil.

mole of uracil could be removed per irradiated cell. If the media contained less than this amount of uracil, the uracil could be quantitatively removed from the medium.

The amount of uracil necessary to cause 50 per cent inhibition of pyrimidine formation by a cell was determined by experiments in which various amounts of uracil were added to minimal media containing ultraviolet-irradiated 6386 cells. The rates of DHO production by the cells were

then measured. The specific conditions were as follows: Cells were first grown in minimal medium plus uracil, harvested, washed, and incubated for 1 hour in minimal medium to allow exhaustion of any "free" pyrimidine in the cell. The cells were again harvested, washed, suspended in minimal salt solution, and ultraviolet-irradiated. Aliquots were then added to media containing known amounts of uracil and the concentrations of cells were determined by turbidity measurement. Determination of the amount of DHO production between 1 and 2 hours after addition of cells to the uracil media was measured. This time interval was found to be the most satisfactory, since about 40 minutes were required for complete absorption of uracil from the medium and stabilization of the rate of DHO formation by the cells. Also, about 2 to 2.5 hours after the start of the experiment the markedly decreased rate of DHO production mentioned above became apparent, while at the same time some release of uracil inhibition caused an increase in the inhibited rates relative to the non-inhibited ones. The latter effect might be a result of a slight residual nucleic acid synthesis.

The results of such an experiment can be seen in Fig. 4, where, at a concentration of 6.2×10^8 cells per ml., uracil at an initial concentration of about 3.4×10^{-6} M depressed DHO formation to one-half of the uninhibited rate. The concentration of pyrimidine in the cell to give 50 per cent inhibition would thus be about 5×10^{-3} M (assuming a unit cell volume of 10^{-12} ml.). This value is only approximate, since the number of metabolically active cells, the cell volume, and the fraction of uracil in the cell in a form not bound (as nucleic acid) are known only approximately. Further, the actual inhibitor might be a free base, riboside, ribotide, or deoxyribotide of either uracil or cytosine. The ratio of these forms in the cell and also the concentration of substrate at the site of inhibition are unknown; hence no value can be calculated for an enzyme inhibition constant. The value found might be expected to agree within a factor of 10 with the true inhibition constant, however.

Inhibition in Vitro of Ureidosuccinic Synthetase—The studies *in vivo* had shown that the inhibitor appeared to act at or before the step of biosynthesis of US, and that later interconversions of pyrimidine intermediates were not affected by the inhibitor. Further, US has been shown to be formed enzymatically from aspartic acid and carbamyl phosphate (11, 8), and it was felt that synthesis of neither the aspartic acid nor the carbamyl phosphate was the site of action of the inhibitor. This feeling was held because carbamyl phosphate, which appears to be necessary for arginine biosynthesis (11), and aspartic acid would both be necessary for protein formation, yet there was no inhibition of protein synthesis when the mutant was grown with uracil. Under such conditions US, DHO, and OA were not produced. Therefore, it appeared likely that the site of the inhibition would be the formation of US itself.

Experiments *in vitro* were carried out as described (8) to determine the rates of US formation under the influence of various possible inhibitors. As shown in Table I, uracil, uridine, uridine-5′-phosphate, and cytosine had no observed inhibitory effects. Cytidine and especially cytidine-5′-phosphate, however, had quite large inhibitory effects. The inhibition appears to be competitive with respect to both carbamyl phosphate and aspartic acid. Against carbamyl phosphate, the inhibition constant, K_i,

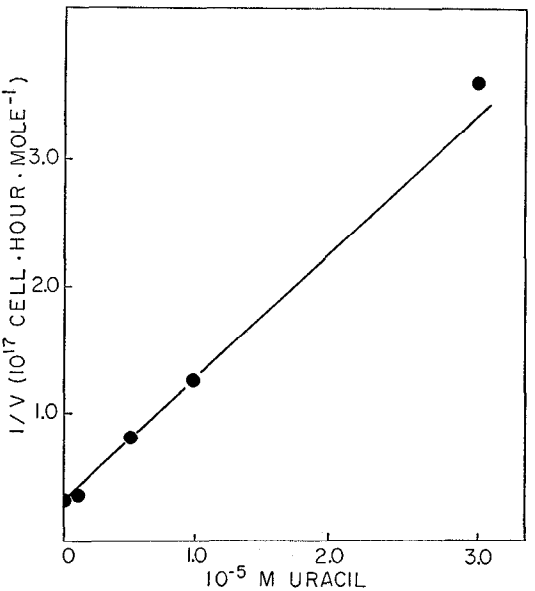

FIG. 4. Uracil inhibition of DHO production by ultraviolet-irradiated cells of *E. coli* 6386. Details in the text. *E. coli* mutant 6386 cells ultraviolet-irradiated for 3.0 minutes were suspended at 6.2×10^8 cells per ml. in minimal medium containing uracil at the concentration shown. The inverse of the per cell rate of DHO production between 1.0 and 2.0 hours after start of incubation is plotted in the graph.

of cytidylic acid was 6×10^{-3} M, and K_i of cytidine was about 8×10^{-3} M (Fig. 5). The results with cytidine as an inhibitor were rather variable, however. It would be reasonable to suspect that cytidine-5′-phosphate (or a derivative) is the true inhibitor *in vivo*, for it is a more effective inhibitor and also might be expected to remain in the cell owing to its negative charge. The reaction appeared to be only slightly competitive with respect to aspartic acid concentration ($K_i \sim 0.01$ M), but the fraction of release of inhibition by either substrate cannot be determined very accurately. The effect of phosphate ion on the reaction is also unknown.

Metabolic Studies—With the finding that an end-product of biosynthesis apparently inhibited the initial step of a whole pathway, it became of in-

terest to determine how wide-spread this phenomenon might be. It has been noticed that when the pyrimidine-requiring mutants exhaust their pyrimidine growth factor, growth, protein synthesis, and nucleic acid synthesis are almost completely stopped (12), but, as shown here, production of the pyrimidine intermediates continues at the rate normal for pyrimidine synthesis of growing, wild type cells. If synthesis of many other metabolites continued at the same rate as in growing cells, the fraction of carbon and nitrogen from the medium converted to the pyrimidine intermediates should be about normal. If other syntheses in the cell were

TABLE I

Effect of Pyrimidines on Rate of US Formation in Vitro

Inhibitor	Concentration	Rate of US formation (moles per hr. per 10^{16} cells)
	M	
None		0.96
Uracil	0.04	0.96
Uridine	0.02	0.88
Uridine-5′-phosphate	0.02	1.00
Cytosine	0.04	0.92
Cytidine	0.02	0.60
Cytidine-5′-phosphate	0.015	0.48

Conditions—The reaction mixtures contained 0.1 M potassium phosphate buffer, pH 6.5, 0.005 M $MgCl_2$, 0.01 M carbamyl phosphate, 0.02 M C^{14}-DL-aspartic acid, extract of 2.5×10^9 *E. coli*, strain B, cells per ml. of reaction, and the inhibitor above carefully neutralized to pH 6.5, if necessary. The reaction mixtures were warmed to 37° before addition of extract, and the reactions were stopped by passage of aliquots through Dowex 50 (acid form) columns. Samples were directly plated and counted for C^{14} and corrected for controls as described (8).

abruptly halted when macromolecule formation ceased, however, many times the normal fraction of carbon and nitrogen should be converted to the unblocked synthesis of pyrimidine intermediates.

From the composition of *E. coli* cells (1), it can be calculated that normally about 4 per cent of the ammonia nitrogen of the medium is converted to the ring nitrogens of the nucleic acid pyrimidines. Table II, however, shows that 50 to 55 per cent of the ammonia (*i.e.* about 13 times the normal amount) could be converted to the pyrimidine intermediates shortly after growth of the mutants was halted by removal of uracil. This result indicates that production of most other nitrogenous compounds can be abruptly halted by the cells. Depending on the previous history of the cells, the per cent incorporation of nitrogen into the pyrimidine precursors actually varied somewhat. If the cells were extensively starved of ammonia

before the experiment, the nitrogen incorporation into pyrimidine intermediates was decreased to about 40 per cent.

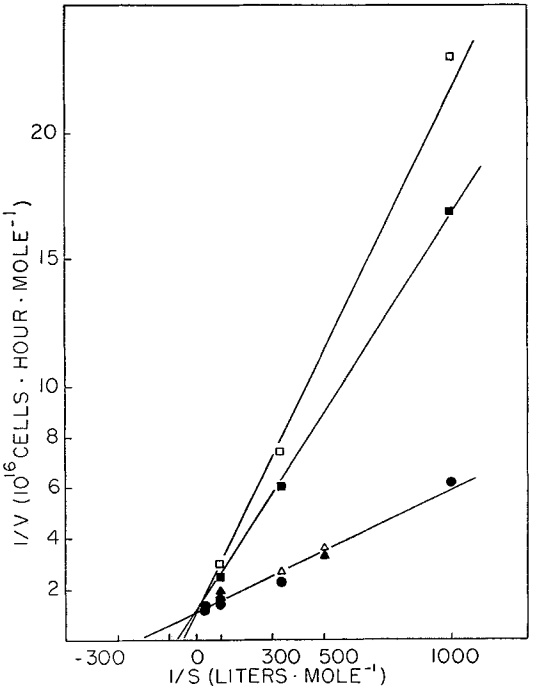

FIG. 5. Rate dependence of US formation on carbamyl phosphate concentration and on inhibitors. The incubation mixture contained 100 μmoles of potassium phosphate buffer, pH 6.5, 5 μmoles of $MgCl_2$, 10 μmoles of C^{14}-DL-aspartic acid, carbamyl phosphate as shown, 20 μmoles of inhibitor (if used), cell-free extract of 2.5×10^9 *E. coli* strain B cells (0.44 mg. of protein) prepared by treatment for 15 minutes in a sonic oscillator. Total volume 1.0 ml. The reaction mixtures were incubated at 37°, and the reaction was halted by passage through Dowex 50 (acid form). The eluate, plus rinse water, was plated, dried, and counted directly in a gas flow Geiger-Müller counter. Correction was made for control samples as described before (8). ●, ▲, and △, no inhibitors (different extracts). □, presence of cytidine-5′-phosphate. ■, presence of cytidine.

If formation of all nitrogenous compounds were self-controlled, it might be expected that 100 per cent of the ammonia would be converted to the non-controlled pyrimidine intermediates, but in practice there will always be slow leakage of all materials from the cell. This leakage would be replaced by synthesis to keep the inhibiting end-product at a high enough concentration to cause further inhibition. Also, the remaining small amount of cell growth (12) would tend to tie up nitrogenous compounds.

Results from the study of carbon incorporation are similar to those with nitrogen, although allowance must be made for oxidation of the carbon source, glycerol, for energy production. The production of pyrimidine intermediates can be determined either by standard chemical assays or by use of C^{14}-glycerol as carbon source with subsequent isotope dilution methods to determine the amounts of products.

TABLE II

Formation of Pyrimidine Intermediates from Limiting Amounts of Ammonia and Glycerol

Mutant	Starting material*		Products in incubation mixture*				
	$(NH_4)_2SO_4$	Glycerol	OA	DHO	US	Total	Fraction incorporated†
550-460	0.30	70	0.100	0.018	0.032	0.150	0.50 (N)
	10.0	1.50	0.080	0.015	0.021	0.116	0.13 (C)
6386	0.30	70		0.083	0.082	0.165	0.55 (N)
	10.0	1.50		0.104	0.076	0.180	0.20 (C)

Conditions—Freshly grown cells were washed, incubated for 45 minutes in minimal medium, harvested, and washed twice in 0.05 M potassium phosphate buffer (pH 7.2) before addition to the test solutions at 1×10^9 per ml. Samples were taken after incubation for 1.5 hours at 37° and centrifuged, and the supernatant solutions were passed through Dowex 50 (acid form) columns. The eluates, diluted 1:1 with the water rinse of the columns, were assayed for OA, DHO, and US as described before (8). The values are the differences between the reaction sample and a control sample lacking the limiting factor in the reaction sample. Samples taken at later incubation times showed no greater total production of intermediates than shown above, although the relative amounts changed.

* All concentrations are $\times 10^{-3}$ M.

† C = carbon; N = ammonia.

Growth of *E. coli* strain B cells in minimal medium containing limiting amounts of glycerol showed that 6.75×10^{-6} mole of glycerol allowed formation of 1.3×10^9 cells containing 8.7×10^{-8} mole of pyrimidine. Thus at the normal rate of pyrimidine biosynthesis, about 2.2 per cent of the glycerol would be expected to pass through the pyrimidine intermediates. It can be calculated further that of the 2.5×10^{-4} gm. per ml. of glycerol carbon, about 1.6×10^{-4} gm. of carbon was incorporated into the cells for a 65 per cent efficiency of carbon utilization.

Tables II and III show that 13 to 20 per cent of the carbon could be converted to the pyrimidine intermediates by the mutant cells. This value is seen to be quite variable, depending on whether the cells were previously starved of glycerol, but is 7 times greater than the 2.2 per cent expected if syntheses of all other carbon compounds continued. The observed value,

however, was far below both the per cent of nitrogen incorporation and the expected limit of 65 per cent carbon incorporation, indicating that synthesis of many carbon compounds, perhaps fats, phospholipides (12), and polysaccharides, still continued in the pyrimidine-deficient cell.

TABLE III

Per Cent Incorporation of C^{14} into US, DHO, and OA by Mutants 6386 and 550-460

Mutant	Condition	Product			
		US	DHO	OA	Total
6386	Non-starved	2.3	8.0		10.3
	Starved of glycerol	2.2	4.6		6.8
550-460	Non-starved	0.81	*	17.3	19
	Starved	0.64	*	11.9	13

Procedure—Freshly grown cells were suspended for 30 minutes at 37° in minimal medium to exhaust the cells of uracil. Part of the cells were then harvested, washed, then suspended for 30 minutes at 37° in minimal salt solution in the absence of glycerol. The cells were harvested and suspended (4×10^8 cells per ml.) in 20 ml. of minimal salt solution; 2.0 μmoles of α-C^{14}-glycerol were added and 0.04 ml. of incubation mixture was removed, plated on platinum dishes and counted in a gas flow Geiger-Müller counter. The cultures were incubated for 1 hour at 37° with rapid swirling; 1 μmole of unlabeled glycerol was added to each flask and incubated 1 hour further. The flask contents were then centrifuged, and carrier non-labeled US, DHO, and OA (with mutant 550-460) were dissolved in 6.0 ml. aliquots of supernatant solution. The solutions were acidified, concentrated *in vacuo* at 45° to about 1.0 ml., crystallized, and the solutions were decanted. The crystals were blotted with paper and similarly recrystallized twice from water. Further crystallization showed no changes in specific activity, except as indicated.

* Samples largely contaminated by C^{14}-orotic acid.

DISCUSSION

The results presented in this paper and the preceding one (8) indicate the means by which pyrimidine biosynthesis may be regulated in *E. coli*. The first observation was that the growth factors uracil and cytosine had inhibitory effects on the formation of ureidosuccinic acid (US) by pyrimidine-requiring *E. coli* mutants. Inhibition of production of dihydroorotic acid (DHO) and orotic acid (OA), later products of the pyrimidine biosynthetic pathway, appears to be only a secondary effect of the earlier metabolic block.

It was reasoned that the inhibition occurred in the formation of US itself, rather than in the formation of its precursors. Demonstration that cytidine and cytidine-5′-phosphate actually were competitive inhibitors of aspartic acid and carbamyl phosphate in the formation of US *in vitro* proved this point. The observed inhibition constants were approximately

the same as those estimated by experiments *in vivo*. The closeness of the agreement of results *in vivo* and *in vitro* is, however, fortuitous.

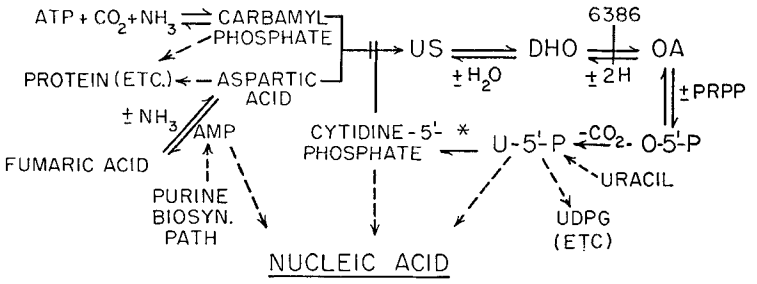

DIAGRAM 1.*, the actual conversion of the uridine derivative to the cytidine derivative was found to occur at the triphosphate level (13).

The last point of evidence was a demonstration of the operation of a regulatory mechanism in the cell. Normally, mutant 6386, which lacks the enzyme dihydroorotic dehydrogenase, can grow rapidly with OA as a growth factor and yet can produce large amounts of the pyrimidine intermediates US and DHO. However, when cells were irradiated with ultraviolet light to halt nucleic acid synthesis and placed in a medium containing OA, the production of the intermediates was quickly inhibited, although the production continued if the cells were in minimal medium. In these ultraviolet-irradiated cells, presumably, the cytidylic acid could not be removed by nucleic acid synthesis as it was being formed from OA, and it served to inhibit US formation as its concentration in the cell increased.

These results support the scheme shown in Diagram 1 for the self-regulated synthesis of pyrimidines by *E. coli*. The individual steps (solid arrows) have all been identified as enzymatic reaction (11, 13, 14, 8).

The operation of the scheme has already been indicated. If an external factor accelerated nucleic acid synthesis, the cytidylic acid concentration of the cell would decrease, thus allowing increased rate of pyrimidine synthesis. If nucleic acid synthesis were slowed or halted, the cytidylic acid (or derivative) concentration would rise and inhibit further pyrimidine biosynthesis. The location of the block is favorable to the cellular economy, since formation of US is essentially an irreversible reaction, while formation of both precursors (aspartate and carbamyl phosphate) involved freely reversible reactions. A block at a later point would thus be inefficient, since, as in the pyrimidine mutants, US would be produced whether it were needed or not, thus wasting energy and metabolites.

A second controlling factor may operate in the system for formation of aspartic acid. Fumaric acid plus ammonia is reported to be converted to aspartic acid in *E. coli* by either aspartase I (15) or aspartase II (16),

the latter enzyme being the most active and being activated by adenylic acid or adenosine. Increase in adenylic acid formation could increase the rate of aspartic acid formation via aspartase II, thus stimulating protein and pyrimidine biosynthesis and thus increasing the rate of nucleic acid synthesis. This effect would complement the stimulation of pyrimidine biosynthesis by decrease in cytidylic acid concentration, for the latter effect merely allows increased US formation if enough aspartic acid and carbamyl phosphate are present.

It seems likely that the rates of many biosynthetic processes in the cell are controlled in a manner similar to that for pyrimidine biosynthesis, since there have been several observations in the literature of end-products blocking their own formation. Probably adenylic or guanylic acid will be found to inhibit the first step in purine biosynthesis that is not freely reversible, and some amino acids will be found similarly to regulate their biosyntheses. The fact that such control mechanisms are wide-spread was indicated here by the metabolism experiments. Gearing of all pathways to the rate of growth would be very important to cells growing slowly in medium deficient in some factor. If a few syntheses were to be uncontrolled, metabolites might be wasted, thus resulting in less cell growth.

These observations may be important for another reason, since it is probable that such normal self-control systems in the cell are the target of inhibitors which are analogues of the natural end-products of such systems. Since such inhibitors are effective, although only traces of the normal metabolite are able to permit growth, the inhibitor must act not by preventing use of the metabolite, but rather by preventing its formation. Knowledge of the exact natural inhibitor of various systems could aid in synthesis of more effective inhibitors. For example, cytidine analogues should, on a theoretical basis, be very effective inhibitors of nucleic acid synthesis (more so than uracil or cytosine derivatives, for example), and this has been found to be true (17).

SUMMARY

1. Low levels (10^{-5} M) of uracil or cytosine, but not of thymine, inhibit formation *in vivo* of the pyrimidine intermediate ureidosuccinic acid in two pyrimidine-requiring mutants of *Escherichia coli*. Formation of the later consecutive intermediates dihydroorotic acid and orotic acid was halted by the absence of ureidosuccinic acid in the cells.

2. The inhibition is not an effect of growth itself, since one mutant (6386) with orotic acid as a growth factor could grow and yet produce ureidosuccinic and dihydroorotic acids.

3. Mutant 6386, in which nucleic acid synthesis was halted by ultraviolet irradiation, produced dihydroorotic acid in the absence, but not in the presence, of orotic acid in the medium.

4. Experiments *in vivo* with the irradiated mutants indicated a concentration of roughly 5×10^{-3} M unbound pyrimidine in the cell necessary to cause half inhibition of US formation.

5. Experiments *in vitro* showed that cytidine and especially cytidine-5′-phosphate were competitive inhibitors with aspartic acid and carbamyl phosphate for ureidosuccinic acid formation. K_i for the cytidylic acid was about 6×10^{-3} M. Uracil, uridine, uridine-5′-phosphate, and cytosine did not inhibit appreciably.

6. Both mutants 6386 and 550-460 in the absence of growth factors synthesized the pyrimidine intermediates at the rate normal for pyrimidine synthesis by wild type cells, but could convert 50 to 55 per cent of the ammonia and 13 to 20 per cent of the glycerol carbon to the intermediates. The normal incorporation into the pyrimidines of growing cells is 4 per cent of the ammonia and 2 per cent of the carbon from the medium.

7. The above facts indicate the existence of a cellular mechanism which links pyrimidine production to the rate of pyrimidine uptake for nucleic acid synthesis. Inhibition by an end-product of its own synthesis appears to be a common control mechanism in the cell.

Addendum—Umbarger (18) has presented preliminary evidence for a feed-back mechanism in isoleucine biosynthesis.

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