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Specificity and Efficiency of Thymidine Incorporation in *Escherichia coli* Lacking Thymidine Phosphorylase

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A mutant of *Escherichia coli* lacking the catabolic enzyme thymidine phosphorylase readily incorporates exogenous thymidine into deoxyribonucleic acid (DNA) even when provided at concentrations as low as 0.2 μg/ml. Incorporation by this prototrophic strain occurs specifically into DNA, since, with radioactively labeled thymidine, (i) more than 98% is incorporated into alkali-stable material, (ii) at least 90% is recovered as thymine after brief formic acid hydrolysis, and (iii) at least 90% is incorporated into material with the buoyant density of DNA. During growth in medium containing thymidine, the bacteria obtain approximately half of their DNA thymines from the exogenous thymidine and half from endogenous synthesis. The thymines and cytosines of DNA can be simultaneously and specifically labeled by thymidine-*2-*^{14}C and uridine-*5-*^{3}H, respectively. The mutant, which does not degrade thymidine, retains the ability to degrade the thymidine analogue 5-bromodeoxyuridine.

In *Escherichia coli*, addition of thymidine to the culture medium results in induction of enzymes which are involved with the catabolism of deoxyribonucleosides and whose structural genes are genetically linked (1, °2, 5, 13). The first enzyme which acts on thymidine, thymidine phosphorylase (EC 2.4.2.4), degrades it to thymine and deoxyribose-1-phosphate. This degradation limits the usefulness of thymidine as a label for deoxyribonucleic acid (DNA) synthesis (17), since thymine is poorly incorporated except in thymine-requiring bacteria (9). Mutant bacteria have been isolated which have lost thymidine phosphorylase activity. These prototrophic strains no longer degrade thymidine and, therefore, incorporate it into cold acid-insoluble material at a constant differential rate for many generations. It was of interest to determine whether thymidine is incorporated only into DNA and not other macromolecules. In this report, experiments with one of these strains, K12SH-28 (11), are described in which the extent and specificity of thymidine incorporation into nucleic acids were determined.

MATERIALS AND METHODS

Strains and medium. *E. coli* strains K12SH and K12SH-28 have been described. Both are prototrophs. K12SH-28 is a single step derivative of K12SH with reduced thymidine phosphorylase activity (11). The medium used was F buffer (23) containing 0.4% glucose. All culture incubations were at 37 C with aeration by shaking.

Incorporation of radioactive nucleosides. Incorporation of radioactive compounds into cold acid-insoluble material was determined by adding a culture sample to an equal volume of cold 10% trichloroacetic acid containing 1 mg of nonradioactive base per ml. After standing at least 30 min on ice, the sample was filtered through a 24-mm membrane filter (Gelman GA-6) and washed twice on the filter with 10 ml of cold water. Dried samples were counted in a liquid scintillation counter in toluene-based scintillation fluid. For measurement of incorporation into alkali-labile and alkali-stable material, culture samples were added to an equal volume of 1 N NaOH containing 1 mg of nonradioactive nucleoside per ml. The samples were incubated for 8 to 12 hr at 37 C, chilled, neutralized with HCl, acidified with trichloroacetic acid, and filtered and washed as above. Alkali-labile counts were determined directly by counting some of the filtrate in a dioxane-based scintillation fluid.

Formic acid hydrolysis. A culture sample containing approximately 10^9 cells (usually 5 ml) was added to an equal volume of cold 10% trichloroacetic acid. After standing on ice for 30 min, the sample was filtered through a Gelman filter (GA-6, 24 mm), and the precipitate was washed on the filter twice with 5 ml of cold 5% trichloroacetic acid and twice with 5 ml of cold water. The filter was then placed in a tube (10 by 75 mm) containing 1.0 ml of 0.5 N NaOH and incubated at 37 C for 15 hr to hydrolyze the ribonucleic acid (RNA; 22). The filter was removed, and the sample was chilled on ice and acidified with 0.1 ml of 5.5 N HCl and 1.1 ml of 10% trichloroacetic acid. After standing on ice, the tube was centrifuged to pellet the precipitate. The super-

natant fluid containing the ribonucleoside monophosphates was saved, and the pellet was dissolved with 0.2 ml of 0.5 N NaOH, chilled, and acidified with HCl and trichloroacetic acid. After centrifugation, the tube was drained and 0.2 ml of 90.7% formic acid was added. The tube was sealed and placed in an oven at 175 C for 15 min (26). The resulting hydrolysate was spotted directly onto thin-layer chromatography plates.

Thin-layer chromatography. All separations were made on poly (ethyleneimine) cellulose-coated plastic sheets prepared as described by Randerath and Randerath (20). Separation of free DNA bases was accomplished by the two-dimensional system of Randerath (19). Duplicate chromatograms were spotted with formic acid hydrolysate (5 to 20 μliters) and a mixture of commercially obtained bases (40 μliters). Separation of nucleoside monophosphates also employed a two-dimensional system (18). The acidified alkaline hydrolysate (20 μliters) was spotted directly onto chromatograms along with a mixture of 2′ and 3′-ribonucleoside monophosphates (10 μliters) and 0.1 M ethylenediaminetetraacetate (10 μliters). For separation of ^{3}H-thymine from ^{3}H-thymidine and ^{3}H-bromouracil from ^{3}H-bromodeoxyuridine, chromatograms were developed to 15 cm with water.

Density gradient centrifugation. A cell lysate was prepared and phenol-extracted. Centrifugation was carried out in CsCl (1.71 g/ml) in a Spinco 50 Ti rotor at 25 C for 48 hr at 144,000 × *g* (12). Fractions (10 drops) were collected into scintillation vials containing glass-fiber filters (Whatman, GF/A), dried, and counted with a toluene-based scintillation fluid.

Nucleoside phosphorylase assay. The composition of the reaction mixture was similar to that described by Razzel (21), except that the deoxyribonucleoside substrate was radioactive and the final volume was 100 μliters. The reaction mixture contained 0.05 M sodium arsenate (*p*H 6), 2×10^{-5} M thymidine-*methyl*-^{3}*H* or 5-bromodeoxyuridine-*6*-^{3}*H*, and toluenized cells (11) in 5×10^{-3} M tris(hydroxymethyl)-aminomethane (*p*H 7.4). The reaction was carried out at 37 C for 30 min and terminated by chilling the tubes and adding 20 μliters of 2 M sodium formate (*p*H 3.4) containing the corresponding pyrimidine (0.03 M) and pyrimidine nucleoside (0.012 M). The mixtures were centrifuged in a Beckman Microfuge for 1 min, and 10 to 20 μliters of the supernatant fluid was then spotted on a thin-layer chromatogram. Specific activity is expressed as nanomoles of pyrimidine nucleoside degraded per minute per milligram of protein.

RESULTS

Effect of thymidine concentration on incorporation. In experiments previously reported (11), ^{14}C-thymidine was employed in the growth medium at a concentration of 5 μg/ml. It was of interest to determine whether lower concentrations could be used with strain K12SH-28 and good incorporation still be obtained. The data in Figure 1 show that the rates of incorporation of ^{14}C-thymidine are essentially identical if the ^{14}C-thymidine concentration in the medium is varied 25-fold, from 0.2 to 5.0 μg/ml. The rate of bacterial growth, determined by culture optical density measurements, is unaffected by the addition of thymidine to these concentrations. Thymidine concentrations in this range, then, do not limit the rate of incorporation.

Specificity of thymidine incorporation. Incorporation of thymidine into RNA was estimated by allowing cells to incorporate thymidine-*methyl*-^{3}*H* for 30 min. Samples were then taken for determination of incorporation into cold acid-insoluble, alkali-labile and alkali-stable material. The results were compared with the incorporation of ^{3}H-uridine into these fractions (Table 1). The amount of ^{3}H-thymidine incorporated into alkali-labile material (primarily RNA) is $<2\%$ the amount of ^{3}H-thymidine incorporated into alkali-stable material (presumably DNA) and is $<0.1\%$ the amount of ^{3}H-uridine incorporated into RNA during the same period. A similar result was obtained when the experiment was performed with thymidine-*2*-^{14}*C*.

To demonstrate that radioactive label from thymidine incorporated into cell material is found only as thymine, K12SH-28 cells were allowed to incorporate ^{14}C-thymidine for 30 min, and samples were taken for a determination of total incorporation (cold acid-insoluble radioactivity) and for formic acid hydrolysis. A 1.0-ml amount of culture incorporated 0.21 nmole of ^{14}C-thymidine. The hydrolyzed culture sample yielded 0.21 nmole of ^{14}C-thymine per 1.0 ml of culture. Thus, within the precision of duplicate determinations ($\pm 5\%$), all of the ^{14}C-thymidine is incorporated into cellular polymers which are stable to alkali and yield ^{14}C-thymine on brief formic acid hydrolysis.

Additional evidence for the specific incorporation of thymidine into DNA was provided by a density gradient centrifugation experiment. A culture of K12SH-28 was allowed to incorporate thymidine-*2*-^{14}*C* for 60 min, and a sample was taken for a determination of total incorporation. It was found that 1.0 ml of culture incorporated 0.13 nmole of ^{14}C-thymidine. A second sample was taken at the same time and a cell lysate was prepared. The lysate was extracted with phenol and dialyzed, and a quantity corresponding to 1.0 ml of culture was centrifuged to equilibrium in CsCl. Fractions were then collected, and the ^{14}C content was determined (Fig. 2). The total radioactivity recovered from the gradient corresponded to 0.14 nmole of ^{14}C-thyimdine, and essentially all of this was found in material banding at the density of DNA (14).

Specific labeling of the cytosines of DNA. Addition of radioactive thymidine to K12SH-28 cultures allows specific labeling of the thymines of DNA, as shown above. The other pyrimidine, cytosine, can be specifically labeled by uridine labeled with tritium on the 5-carbon. The hydrogen atom on the 5-carbon of uridine is removed during its metabolic conversion to thymidine triphosphate (15, 25), and is substituted by a methyl group. A tritium atom at this position in uridine, therefore, should become part of the cytidylic acid residues but not the thymidylic acid residues of DNA. That this is the case was shown by incubating a culture of K12SH-28 with uridine-*5-*^3H for 30 min and hydrolyzing a sample to produce RNA nucleotides and DNA bases. The tritium label is found in both the cytosines and uracils of RNA but only in the cytosines of DNA (Table 2). If there is any incorporation of label into the thymines of DNA, the amount must be <3% of that found in the cytosines of DNA. The presence of both uridine and thymidine in the medium does not influence the incorporation of either into DNA (Table 3, cultures 1 to 3).

Efficiency of incorporation of exogenous thymidine. The fact that the rate of incorporation of ^{14}C-thymidine by cultures of K12SH-28 is independent of the ^{14}C-thymidine concentration in the range 0.2 to 5.0 μg/ml (Fig. 1) could mean that, even at the lowest concentration employed, exogenous thymidine is the source of 100% of the thymines of DNA. That this is not so is suggested by an experiment with 5-fluorodeoxyuridine, an inhibitor of endogenous thymidylate synthesis (8). Comparison of the values for cultures 1 and 4 (Table 3) shows that addition of the analogue (along with adenosine and uridine) to the medium stimulates incorporation of ^{14}C-thymidine by a culture of K12SH-28. (Addition of adenosine and uridine without 5-fluorodeoxyuridine has no effect on incorporation.) This result suggests that endogenous synthesis supplies some of the thymidylate residues of DNA when thymidine alone is added to cultures of this strain. This was also suggested by experiments in which the differential

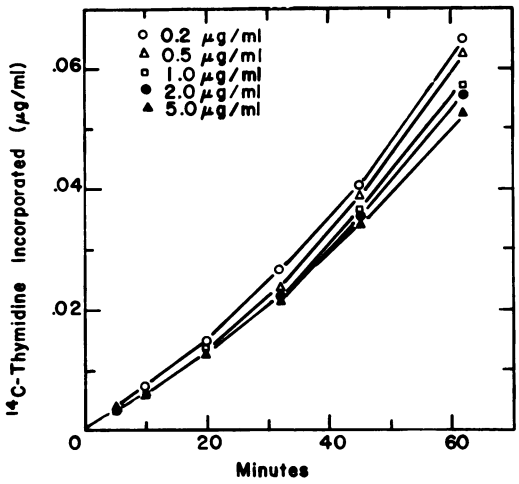

FIG. 1. *Effect of ^{14}C-thymidine concentration on the rate of incorporation by strain K12SH-28. An exponentially growing bacterial culture was added to flasks containing various amounts of thymidine-2-^{14}C (0.04 μc/μg). At intervals, 1.0-ml samples were added to cold 10% trichloroacetic acid.*

TABLE 1. *Incorporation of ^3H-thymidine and ^3H-uridine into RNA and DNA*[a]

Medium supplement	Nucleoside incorporated (nmoles/ml)		
	Acid-insoluble (RNA + DNA)	Alkali-labile (RNA)	Alkali-stable (DNA)
^3H-thymidine	0.16	<0.002	0.17
^3H-uridine	3.7	3.0	0.24

[a] Portions of an exponentially growing culture of strain K12SH-28 were added to flasks containing thymidine-*methyl-*^3H (4.1 nmole/ml final concentration, 0.3 μc/nmole)or uridine-*5-*^3H (41 nmole/ml final concentration, 0.04 μc/nmole). Acid-insoluble, alkali-labile and -stable radioactivities were determined on samples taken at 30 min.

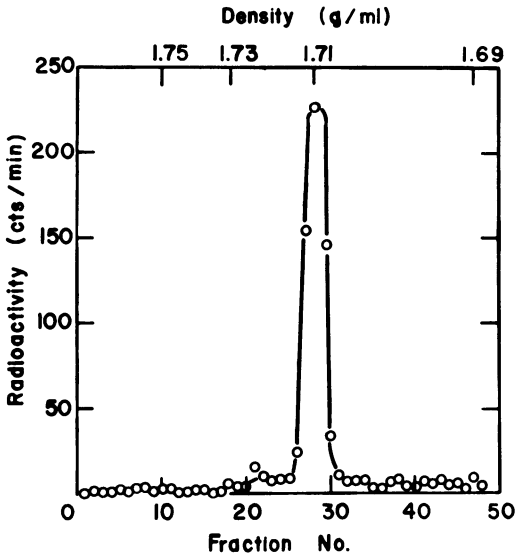

FIG. 2. *CsCl banding of material in K12SH-28 containing radioactivity from ^{14}C-thymidine. Sample preparation and centrifugation are described in the text.*

TABLE 2. *Incorporation of uridine-5-^3H into RNA and DNA bases*[a]

Nucleic acid	Nucleotide or base[b]	Uridine incorporated
		(nmoles/ml)
RNA	AMP	<0.05
	GMP	<0.05
	CMP	1.7
	UMP	1.7
DNA	Adenine	<0.005
	Guanine	<0.005
	Cytosine	0.19
	Thymine	<0.005

[a] Uridine-5-^3H (0.04 μc/nmole) was added to an exponentially growing culture of K12SH-28 at a final concentration of 41 nmoles/ml. Incorporation was terminated after 30 min. Hydrolysis of RNA and DNA, and chromatography of the hydrolysates is described in Materials and Methods.

[b] Nucleotides are the 2'- and 3'-monophosphates produced by alkaline hydrolysis. AMP, adenosine monophosphate; GMP, guanosine monophosphate; CMP, cytidine monophosphate; UMP, uridine monophosphate.

TABLE 3. *Incorporation of nucleosides into DNA*

Culture	Medium supplements[a]	Nucleoside incorporated into DNA[b]
		(nmoles/ml)
1	^{14}C-thymidine	0.15
2	^3H-uridine	0.19
3	^{14}C-thymidine	0.14
	plus ^3H-uridine	0.19
4	^{14}C-thymidine plus uridine, adenosine, and 5-fluorodeoxyuridine	0.31

[a] Cultures 1 to 4: thymidine-2-^{14}C, 4.1 nmoles/ml (0.01 μc/nmole); uridine-5-^3H, 41 nmoles/ml (0.01 μc/nmole). Culture 4: uridine, 200 nmoles/ml; adenosine, 150 nmoles/ml; 5-fluorodeoxyuridine, 19 nmoles/ml.

[b] Portions of an exponentially growing culture of K12SH-28 were added to flasks containing the various compounds. After 30 min, incorporation of radioactivity into alkali-stable, cold acid-insoluble material was determined.

rates of incorporation of thymidine (^3H-thymidine incorporated per increment of culture optical density) by K12SH-28 and a thymidine-requiring derivative isolated by the aminopterin selection technique (16) were compared. The differential rate of incorporation by the latter strain is about 1.6-fold greater than by K12SH-28. Thymidine-requiring derivatives of K12SH-28 can utilize levels of thymidine in the medium as low as the original strain. Thymine is unable to satisfy their growth requirement.

The fraction of the thymines of DNA derived from exogenous thymidine and from endogenous synthesis in K12SH-28 was determined directly by a competition experiment. Cells were grown in medium containing a limiting amount of uniformly labeled ^{14}C-glucose, with and without a thymidine supplement. When growth terminated on exhaustion of the ^{14}C-glucose, samples were taken, and radioactivity in the four bases was determined. The results were compared with the effect of a uridine supplement (Table 4). Growth of the cells in medium containing nonradioactive thymidine results in approximately a 55% reduction in the radioactivity found in the thymines of DNA. That is, approximately 55% of the thymines of DNA are derived from exogenous thymidine and approximately 45% from endogenous synthesis. Uridine competes more efficiently with endogenous synthesis for incorporation into the thymines of DNA (Table 4).

Limited incorporation of 5-bromodeoxyuridine. Since the thymidine analogue 5-bromodeoxyuridine has been widely used as a density label for DNA and can be used to facilitate isolation of bacterial mutants (3), the ability of K12SH-28 to incorporate 5-bromodeoxyuridine was examined. Figure 3A shows the incorporation values obtained when ^3H-5-bromodeoxyuridine is added to a culture of K12SH-28 previously grown in thymidine-supplemented minimal medium. At the cell density employed, incorporation

TABLE 4. *Competition of thymidine and uridine with ^{14}C-glucose for entry into DNA bases*[a]

Medium supplement	Radioactivity in DNA bases (relative to adenine)			
	Adenine	Guanine	Cytosine	Thymine
None	1.0	0.86	0.82	1.1
Thymidine	1.0	0.81	0.90	*0.50*
Uridine	1.0	0.80	*0.04*	*0.24*

[a] Washed cells of strain K12SH-28 were inoculated at 10^5 cells/ml into flasks containing 5 ml of F buffer supplemented with 120 μg of uniformly labeled ^{14}C-glucose (0.12 μc/μg) per ml and, where indicated, 1.0 μg of thymidine or 10 μg uridine per ml. Cultures were incubated until the glucose was exhausted (10^8 cells/ml). The concentrations of thymidine and uridine are in about 10-fold excess for the quantity of cells produced. The three cultures were then processed for formic acid hydrolysis, hydrolyzed, and chromatographed. Significant differences are shown in italics.

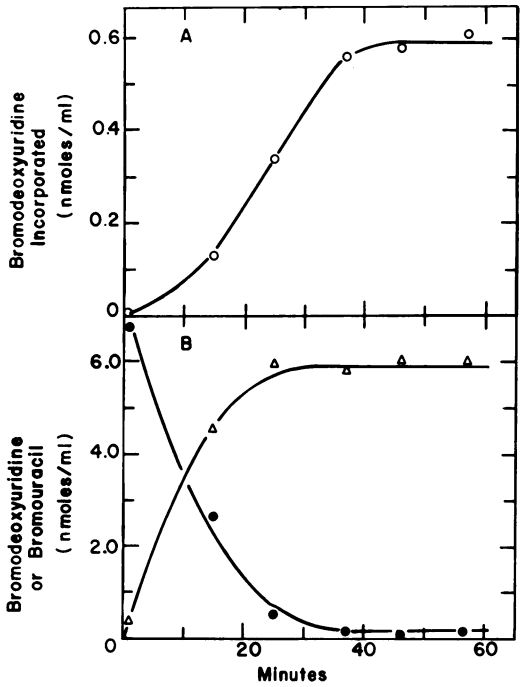

FIG. 3. *Incorporation of ^{3}H-bromodeoxyuridine and its degradation to ^{3}H-bromouracil. 5-Bromodeoxyruidine-6-^{3}H (0.05 μc/nmole) was added to an exponentially growing culture of K12SH-28. Samples were added to cold trichloroacetic acid for determination of incorporated radioactivity. Additional samples (100 μliters) were added to 20 μliters of 2 M sodium formate, pH 3.4. The mixtures were centrifuged for 1 min in a Beckman Microfuge, and 10 μliters of the supernatant fluid was spotted on thin-layer chromatograms, along with 10 μliters containing bromodeoxyuridine (0.5 mg/ml) and bromouracil(1.25 mg/ml) and 10 μliters of 0.1 M ethylenediaminetetraacetate.*

terminated after about 30 min. One explanation of this would be that bromodeoxyuridine, unlike thymidine, is still rapidly degraded, as in wild-type *E. coli*. The data in Fig. 3B show that ^{3}H-bromodeoxyuridine rapidly disappears from the culture and is replaced by ^{3}H-bromouracil, which is not incorporated into DNA.

Degradation of bromodeoxyuridine by K12SH-28 could mean that the strain possesses an unaltered phosphorylase activity for this deoxyribonucleoside. Phosphorylase activities in K12SH-28 and its wild-type parental strain, K12SH, were examined by using both thymidine and bromodeoxyuridine as substrates. Cultures were grown for several generations in minimal medium containing 1 mg of deoxyadenosine per ml as inducer (13). As reported previously (11), activity with thymidine is greatly reduced in K12SH-28, being <0.5% of the wild-type value (Table 5). In the assay employed, activity with bromodeoxyuridine is also greatly reduced (about 0.5% of the wild-type value). Uridine phosphorylase (EC 2.4.2.3) activity, an uninducible activity (21) in both strains, is shown for comparison. Phosphorylase activity for bromodeoxyuridine as well as for thymidine is reduced in K12SH-28, and some other explanation for the in vivo degradation of bromodeoxyuridine must be found.

DISCUSSION

The mutation affecting thymidine phosphorylase activity in strain K12SH-28 has been mapped by transduction (10). The strain is capable of producing normal induced levels of deoxyriboaldolase (EC 4.1.2.4) and phosphodeoxyribomutase whose structural genes appear to be linked to that for thymidine phosphorylase (13). Revertants, capable of growth on thymidine as a source of carbon and energy (and containing thymidine phosphorylase activity), are readily obtained. These facts suggest that K12SH-28 possesses a single mutation in the structural gene for thymidine phosphorylase.

Taken together, the results of several experiments show that radioactively labeled thymidine is specifically incorporated into DNA and not RNA or other macromolecules. First, more than 98% of cold acid-insoluble radioactivity is stable to alkali. Thus, less than 2% of the label could be in RNA (24). Second, more than 90% of the incorporated radioactivity is recovered as thymine after brief formic acid hydrolysis (26). And third, more than 90% of the incorporated radioactivity is found in material which bands in CsCl at the density of *E. coli* DNA (14).

As expected, labeled thymidine is incorporated only into the thymines of DNA. The other pyrimidine in DNA, cytosine, can be specifically labeled by employing uridine-5-^{3}H in the medium. At least two of the DNA bases, then, can be

TABLE 5. *Phosphorylase activity of cells induced with deoxyadenosine*[a]

Substrate	Specific activity[b]	
	Strain K12SH	Strain K12SH-28
^{3}H-thymidine	150	<0.4
^{3}H-bromodeoxyuridine	340	1.7
^{3}H-uridine	5.3	4.5

[a] Cells were grown for three generations in medium containing 1 mg of deoxyadenosine (13) per ml, washed, and assayed.

[b] Expressed as nanomoles per minute per milligram.

easily and specifically labeled. It should be possible to specifically label the adenines of DNA by using adenosine-2-^{3}H.

The stimulation of ^{14}C-thymidine incorporation by 5-fluorodeoxyuridine, the increased incorporation by a thymidine-requiring derivative, and the competition for incorporation into DNA of exogenous thymidine with ^{14}C-thymidine compounds made endogenously from ^{14}C-glucose show that only 50 to 60% of the DNA thymines are derived from exogenous thymidine by K12SH-28. A similar result was obtained by Cannon and Breitman (7) for incorporation of exogenous thymidine monophosphate. In the range of thymidine concentrations used in these experiments, the differential rate of incorporation by K12SH-28 is essentially unaffected. Whether endogenous thymidine triphosphate synthesis, which appears to occur by two pathways (15), is affected by the presence of exogenous thymidine is unknown. One explanation for the inefficient utilization of exogenous thymidine would be that addition of thymidine to the medium results in a large reduction in the rate of endogenous thymidine triphosphate formation from deoxyuridine monophosphate but not from deoxycytidine triphosphate (15). A similar effect of added uridine could explain its efficient incorporation into DNA cytosines but not DNA thymines (Table 4).

Budman and Pardee (6) found that addition of deoxyadenosine to the culture medium did not promote thymine incorporation by K12SH-28 as it does in wild-type *E. coli* (4). Their result suggested that thymidine phosphorylase catalyzes the conversion of thymine to thymidine under appropriate in vivo conditions. This conclusion is supported by the observation that a thymidine-requiring strain derived from K12SH-28 is unable to utilize thymine to fulfill the growth requirement. Revertants of this double mutant capable of growth on thymidine as a source of carbon and energy, and which possess thymidine phosphorylase activity, are able to use thymine to meet their growth requirement.

Rachmeler, Gerhart, and Rosner (17) provided evidence which suggests that a single inducible phosphorylase acts on both thymidine and 5-bromodeoxyuridine. It was surprising, therefore, to find that K12SH-28 retains the ability to rapidly degrade it. Nevertheless, K12SH-28 shows a greatly reduced phosphorylase activity for both deoxyribonucleosides. Several explanations for the in vivo degradation of bromodeoxyuridine but not thymidine by K12SH-28 are plausible. *E. coli* may possess a "bromodeoxyuridine" phosphorylase which is either not induced or not active under the conditions employed in the enzyme assay experiment. A likely explanation is that the thymidine phosphorylase in K12SH-28 has a greatly increased Michaelis constant for thymidine and bromodeoxyuridine. If the Michaelis constant for bromodeoxyuridine is, however, somewhat lower than that for thymidine, sufficient intracellular concentrations may occur to allow rapid phosphorolysis of bromodeoxyuridine but not thymidine.

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LITERATURE CITED

1. Alikhanian, S. I., T. S. Iljina, E. S. Kaliaeva, S. V. Kameneva, and V. V. Sukhodolec. 1966. A genetical study of thymineless mutants of *E. coli* K12. Genet. Res. 8:83–100.
2. Barth, P. T., I. R. Beacham, S. I. Ahmad, and R. H. Pritchard. 1968. The inducer of the deoxynucleoside phosphorylases and deoxyriboaldolase in *Escherichia coli*. Biochim. Biophys. Acta 161:554–557.
3. Bonhoeffer, F., and H. Schaller. 1965. A method for the selective enrichment of mutants based on the high UV sensitivity of DNA containing 5-bromouracil. Biochem. Biophys. Res. Commun. 20:93–97.
4. Boyce, R. P., and R. B. Setlow. 1962. A simple method of increasing the incorporation of thymidine into the deoxyribonucleic acid of *E. coli*. Biochim. Biophys. Acta 61:618–620.
5. Breitman, T. R., and R. M. Bradford. 1968. Inability of low thymine-requiring mutants of *Escherichia coli* lacking phosphodeoxyribomutase to be induced for deoxythymidine phosphorylase and deoxyriboaldolase. J. Bacteriol. 95:2434–2435.
6. Budman, D. R., and A. B. Pardee. 1967. Thymidine and thymine incorporation into deoxyribonucleic acid: inhibition and repression by uridine of thymidine phosphorylase of *Escherichia coli*. J. Bacteriol. 94:1546–1550.
7. Cannon, W. D., Jr. and T. R. Breitman. 1967. Control of deoxyribonucleotide biosynthesis in *Escherichia coli*. I. Decrease of pyrimidine deoxyribonucleotide biosynthesis *in vivo* in the presence of deoxythymidylate. Biochemistry 6:810–816.
8. Cohen, S. S., J. G. Flaks, H. D. Barner, M. R. Loeb, and J. Lichtenstein. 1958. The mode of action of 5-fluorouracil and its derivatives. Proc. Nat. Acad. Sci. U. S. A. 44:1004–1012.
9. Crawford, L. V. 1958. Thymine metabolism in strains of *Escherichia coli*. Biochim. Biophys. Acta 30:428–429.
10. Dale, B., and G. R. Greenberg. 1967. Genetic mapping of a mutation in *Escherichia coli* showing reduced activity of thymidine phosphorylase. J. Bacteriol. 94:778–779.
11. Fangman, W. L., and A. Novick. 1966. Mutant bacteria showing efficient utilization of thymidine. J. Bacteriol. 91:2390–2391.
12. Flamm, W. G., H. E. Bond, and H. E. Burr. 1966. Density-gradient centrifugation of DNA in a fixed-angle rotor. A higher order of resolution. Biochim. Biophys. Acta 129:310–319.
13. Lomax, M. S., and G. R. Greenberg. 1968. Characteristics of the *deo* operon: role of thymine utilization and sensitivity to deoxyribonucleosides. J. Bacteriol. 96:501–514.
14. Meselson, M., and F. W. Stahl. 1958. The replication of DNA in *Escherichia coli*. Proc. Nat. Acad. Sci. U. S. A. 44:671–682.

15. Neuhard, J. 1968. Pyrimidine nucleotide metabolism and pathways of thymidine triphosphate biosynthesis in *Salmonella typhimurium*. J. Bacteriol. 96:1519–1527.
16. Okada, T., K. Yanagisawa, and F. J. Ryan. 1961. A method of securing thymineless mutants from strains of *E. coli*. Z. Vererbungsl. 98:403–412.
17. Rachmeler, M., J. Gerhart, and J. Rosner. 1961. Limited thymidine uptake in *Escherichia coli* due to an inducible thymidine phosphorylase. Biochim. Biophys. Acta 49:222–225.
18. Randerath, K. 1964. Separation of the constituent nucleotides of nucleic acids on ion-exchange thin-layers. Experientia (Basle) 20:406–407.
19. Randerath, K. 1965. Two-dimensional separation of nucleic acid bases on cellulose layers. Nature 205:908.
20. Randerath, K., and E. Randerath. 1966. Ion-exchange thin-layer chromatography. XV. Preparation, properties, and applications of paper-like PEI-cellulose sheets. J. Chromatog. 22:110–117.
21. Razzel, W. E. 1967. Pyrimidine nucleoside and deoxynucleoside phosphorylases, p. 118–125. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12, part A. Academic Press Inc., New York.
22. Roodyn, D. B., and H. G. Mandel. 1960. A simple membrane fractionation method for determining the distribution of radioactivity in chemical fractions of *Bacillus cereus*. Biochim. Biophys. Acta 41:80–88.
23. Sadler, J. R., and A. Novick. 1965. The properties of repressor and the kinetics of its action. J. Mol. Biol. 12:305–327.
24. Schmidt, G., and S. J. Thannhauser. 1945. A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. J. Biol. Chem. 161:83–89.
25. Wahba, A. J., and M. Friedkin. 1962. The enzymatic synthesis of thymidylate. I. Early steps in the purification of thymidylate synthetase of *Escherichia coli*. J. Biol. Chem. 237: 3794–3801.
26. Wyatt, G. R., and S. S. Cohen. 1953. The bases of the nucleic acids of some bacterial and animal viruses: the occurrence of 5-hydroxymethylcytosine. Biochem. J. 55:774–782.