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Characteristics of the *deo* Operon: Role in Thymine Utilization and Sensitivity to Deoxyribonucleosides[1]

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Inability to grow on deoxyribonucleosides as the sole carbon source is characteristic of *deo* mutants of *Escherichia coli*. Growth of *deoC* mutants, which lack deoxyribose 5-phosphate aldolase, is reversibly inhibited by deoxyribonucleosides through inhibition of respiration. By contrast, *deoB* mutants are not sensitive to deoxyribonucleosides, and deoxyribose 5-phosphate aldolase and thymidine phosphorylase are present at normal levels but are not inducible by thymidine. Organisms with the genotype *deoB*$^-$ *thy*$^-$ or *deoC*$^-$ *thy*$^-$ are able to grow on low levels of thymine, whereas *deoB*$^+$ *thy*$^-$ or *deoC*$^+$ *thy*$^-$ strains require high levels of thymine for growth. The *deoB* and *deoC* mutations are transducible with and map on the counterclockwise side of the threonine marker. They are closely linked to *deoA*, a gene determining thymidine phosphorylase. Merodiploids heterozygous for either the *deoB* or *deoC* genes are resistant to deoxyribonucleosides and, in combination with the *thy* mutation, require high levels of thymine for growth. Cultures of *thy*$^+$ *deoC*$^-$ mutants are inhibited by thymidine until this compound has been completely degraded and excreted as deoxyribose and thymine, whereupon growth promptly resumes at a normal rate. The inhibition of respiration in *deoC* strains and the induction of thymidine phosphorylase and deoxyribose 5-phosphate aldolase in the wild-type organism are considered to result from the accumulation of deoxyribose 5-phosphate.

At least three enzymes appear to be required for the catabolism of thymidine by *Escherichia coli*: thymidine (TdR) phosphorylase (thymidine: orthophosphate deoxyribosyltransferase, EC 2.4.2.4); 1,5-phosphodeoxyribomutase (PDM); and deoxyribose 5-phosphate (dR-5-P) aldolase (2-deoxy-D-ribose 5-phosphate acetaldehyde lyase, EC 4.1.2.4) (6, 18, 28, 29, 33).

$$\text{TdR} \xrightleftharpoons{\text{TdR phosphorylase}} \text{thymine} + \text{dR}-1-\text{P}$$

$$\text{dR}-1-\text{P} \xrightleftharpoons{\text{PDM}} \text{dR}-5-\text{P} \xrightleftharpoons{\text{dR-5-P aldolase}} \text{glyceraldehyde 3-phosphate} + \text{acetaldehyde}$$

The three enzymes are all reported to be inducible by deoxyribonucleosides (5, 7, 30; H. O. Kammen, Federation Proc. **26**:809, 1967).

Thymine-requiring (*thy*) mutants isolated by means of the aminopterin selection procedure of Akada et al. (26) lack the enzyme thymidylate synthetase which converts deoxyuridylate (dUMP) to thymidylate (dTMP), and these mutants show a high thymine requirement for growth, i.e., 20 μg/ml or more. Recently, investigators in several laboratories have shown that mutants with a low thymine requirement, i.e., 1 to 2 μg/ml, can be selected directly from high thymine-requiring *thy* strains and that such low thymine-requiring mutants are actually double mutants. Alikhanian and co-workers (2) and Okada (25) have mapped the mutation to a low thymine requirement near the *thr* locus. Breitman and Bradford (5) have provided evidence that a low thymine-requiring *thy*$^-$ strain of *E. coli* 15 (70V3) (4) lacks dR-5-P aldolase. They have ascribed the low thymine requirement of this strain to an accumulation of dR-1-P, which is a cosubstrate in the conversion of thymine to TdR. These authors have suggested this compound is normally the limiting factor in thymine utilization.

This paper reports studies of two genes, *deoB* and *deoC*, and provides evidence that they are involved in determining two of the enzymes of the pathway described above. The *deo* designation

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has been adopted to indicate a gene determining an enzyme necessary for growth on deoxythymidine or deoxyuridine as the sole source of carbon (10). Phenotypically, organisms carrying mutations in either gene B or C and in the structural gene (*thy*) for thymidylate synthetase, i.e., with the genotypes *deoB*$^-$ *thy*$^-$ or *deoC*$^-$ *thy*$^-$, exhibit a low thymine requirement for growth, whereas *deoB*$^+$ *thy*$^-$ or *deoC*$^+$ *thy*$^-$ strains require high thymine. The growth of strains carrying the *deoC*$^-$ mutation is inhibited by deoxyribonucleosides as a result of the inhibition of respiration, and TdR phosphorylase is inducible in these mutants. With *deoB*$^-$ strains, which are not sensitive to deoxyribonucleosides, neither TdR phosphorylase nor dR-5-P aldolase is inducible. Recently, a mutant organism deficient in TdR phosphorylase was isolated by Fangman and Novick (12), and the mutation (*deoA*$^-$) was then shown to be cotransducible with the *thr* marker by Dale and Greenberg (10).

The present investigation relates the properties of *deoB* and *deoC* mutants to the genetic findings of Alikhanian and co-workers and of Okada and to the biochemical studies by Breitman and Bradford on low thymine-requiring *thy* mutants. dR-5-P aldolase has been shown to be absent in *deoC* mutants but present in *deoB* mutants. Evidence is presented that both the inhibitory effect of deoxyribonucleosides in *deoC* mutants and the induction of TdR phosphorylase and dR-5-P aldolase by deoxyribonucleosides in wild-type cultures result from the accumulation of dR-5-P. In simultaneous studies, Breitman and Bradford (7; *personal communication*) have found low thymine-requiring mutants of *E. coli* strain 15 (*thy*$^-$) lacking phosphodeoxyribomutase which were not inducible. These workers have also concluded that dR-5-P was the inducer.

MATERIALS AND METHODS

Biological materials. The bacterial strains employed in this study and their complete phenotypes are presented in Table 1. The transducing phage P1bt (15) was kindly provided by E. Englesberg, and *Shigella* 16, the indicator bacterium for this phage, was obtained from R. L. Somerville.

Media. The following media were used in this study: nutrient broth (0.8% Difco Nutrient Broth and 0.5% NaCl); L broth, L agar, and soft agar (22); and the minimal medium of Vogel and Bonner (36) containing 0.2% glucose as the carbon source. Minimal medium was supplemented with 10 μg of thymine per ml for low thymine requirers or 50 μg of thymine per ml for high thymine requirers and L-amino acids (each at 20 μg/ml) as required.

Culture methods. Bacterial stocks were maintained on nutrient agar slants supplemented with 50 μg of thymine per ml for *thy*$^-$ strains. Overnight nutrient broth cultures were centrifuged and washed once with an equal volume of saline before being used to inoculate prewarmed minimal medium. Cultures were incubated at 37 C on a New Brunswick gyratory shaker in Erlenmeyer flasks having Klett colorimeter tube side arms. Growth was monitored turbidimetrically with a no. 66 filter or by measuring the absorbance of samples at 590 mμ with a Beckman DU monochromator fitted with a Gilford absorbance converter. A reading of 10 Klett units or 0.12 A_{590} represented approximately 10^8 cells/ml in minimal medium.

Mating conditions. The procedure described by de Haan and Gross (11) was followed for conjugation experiments.

Transfer of episome. Suitable samples of cultures of donor and recipient strains growing exponentially in nutrient broth were mixed to obtain a cell density of 5×10^8 and a ratio of 10 F$^-$ to 1 F$'$. The frequency of transfer of episomal markers was determined by diluting and plating the culture on selective media after incubation at 37 C for 60 min. To determine the kinetics of transfer of episomal markers, samples of the mating mixture were diluted into 10 ml of ice-cold diluting medium at 5-min intervals. Mating pairs were disrupted by vigorous agitation on a Vortex mixer for 1 min. The mixture was plated on selective media as described above.

Transduction experiments. Transducing lysates of phage P1bt were prepared by confluent lysis on L-agar plates (22). Lysates were stored in the cold over chloroform and were titered on L-agar plates with *Shigella* 16 as the indicator bacterium. Transductions were performed as described by Lennox (22). Recombinants were usually streaked on homologous media to obtain single-colony isolates before testing for unselected markers. To test for nutritional markers, single colonies were picked with sterile wooden sticks and transferred to homologous media and test plates in an array. Plates were incubated at 37 C and examined for growth after 1 day. To test for sensitivity to deoxyribonucleosides, single colonies were suspended in 0.5 ml of saline and streaked on selective agar plates containing thymidine (200 μg/ml). Resistant colonies produced streaks which were visible within 24 hr, whereas deoxyribonucleoside-sensitive isolates were not visible for 2 to 3 days.

Disc diffusion assay for sensitivity to deoxyribonucleosides. This assay was a modification of the procedure of Allen and Yanofsky (3) for quantitating the sensitivity of various strains to inhibitors. Approximately 10^6 cells in 3.0 ml of minimal top-layer agar were poured onto minimal agar plates supplemented with any required nutrients except thymine. A 0.1-ml sample of the test solution was pipetted onto a sterile filter-paper disc (13 mm in diameter) placed at the center of the plate. Compounds to be tested for their effect on the inhibition by thymidine were incorporated into the top-layer agar. After a 24- or 48-hr incubation at 37 C, the distance from the edge of the disc to the line of growth was measured.

Oxygen-uptake studies. Bacterial cultures grown to a concentration of about 4×10^8 cells/ml in glucose-minimal salts medium were maintained in ice until use. Portions (1 ml) of the culture were transferred to the main compartment of Warburg flasks containing 0.1 ml of various nucleosides in the side arms and containing alkali in the center well. The uptake

TABLE 1. *Bacterial strains*[a]

Strain	*deoC*	*deoB*	*deoA*	*thy*	*arg*	*pyrA*	*his*	*leu*	*met*	*pro*	*thr*	*trp*	*ara*	*gal*	*lac*	*str*	*T6*	Sex
B[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S	S	F⁻
B3 (72)[c]	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	S	S	F⁻
B/r-62[d]	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	S	S	F⁻
B307c[d]	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	S	S	F⁻
B840c[d]	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	S	S	F⁻
HB45[e]	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	R	S	F⁻
201[f]	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	S	S	F⁻
277[g]	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	R	R	F⁻
296[h]	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S	S	F⁻
297[i]	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	S	S	F⁻
300[j]	+	+	+	+	+	−	+	−	+	+	−	+	−	+	+	S	S	F⁻
302[k]	+	−	+	−	−	+	−	−	−	−	−	−	−	−	−	R	R	F⁻
303[k]	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	R	R	F⁻
310[l]	−	+	+	−	+	+	−	−	−	−	−	−	−	−	−	R	R	F⁻
311	+	+	+	+	+	−	+	+	+	+	−	+	−	+	+	S	S	F⁻
315[m]	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S	S	F⁻
318	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	R	R	F⁻
322[n]	−	+	+	−	+	−	−	+	−	−	−	−	−	−	−	R	R	F⁻
339[o]	−	+	+	+	−	+	−	−	−	−	−	−	−	−	−	R	R	F⁻
388[p]	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	S	S	F⁻
F'araB24/ araB24[q]	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	S	S	F'

[a] The following symbols are used: +, wild type; −, inability to synthesize or utilize; S, sensitivity; R, resistance; *deo*, deoxyribonucleoside; *thy*, thymine; *ara*, arabinose; *gal*, galactose; *lac*, lactose; *arg*, arginine; *pyrA*, structural gene for carbamyl phosphate synthetase (27); *his*, histidine; *leu*, leucine; *met*, methionine; *pro*, proline; *thr*, threonine; *trp*, tryptophan; *str*, streptomycin; *T6*, bacteriophage T6.

[b] The prototrophic *E. coli* B strain from which all mutants were derived was obtained from J. Spizizen in 1957.

[c] Originally isolated by S. Brenner and obtained from N. Melechen, St. Louis University, in 1965.

[d] These strains were kindly provided by F. Kaudewitz (21). B307c and B840c were isolated after treatment of *E. coli* B with nitrous acid; B/r-62 was induced by decay of ^{32}P incorporated into cells of *E. coli* B/r. The mutants were identified by replica plating; no other selection procedure was involved in the isolation of these mutants (F. Kaudewitz, *personal communication*).

[e] A B/r strain which was obtained from H. Boyer.

[f] A *deoB*⁻ *thy*⁻ derivative isolated by plating a *deoB*⁺ *thy*⁻ mutant (strain 104) on a suboptimal level of thymine. Strain 104 was isolated by means of a modification of the procedure of Okada et al. (26) which employs trimethoprim rather than aminopterin (34) as the selective agent. The procedure was modified further by selecting mutants directly on agar plates containing trimethoprim (5 μg/ml) and thymine (20 μg/ml) rather than in liquid culture (B. Dale and G. R. Greenberg, *unpublished procedure*).

[g] T6R derivative of HB45 obtained by direct selection.

[h] Constructed by means of the cross described in Table 6, in which B840c was the donor.

[i] A *thr*⁻ mutation induced by ultraviolet irradiation of *E. coli* B and isolated by the penicillin selection procedure of Gorini and Kaufman (14).

[j] A *thr pyrA araA13 leu-1* derivative of strain B/r which was obtained from R. Helling, Department of Botany, The University of Michigan.

[k] Obtained by plating strain 277 (10^6 cells) on minimal medium containing all the required amino acids plus thymine (5 μg/ml) and trimethoprim (5 μg/ml).

[l] An *arg*⁺ derivative of 303 produced by transduction.

[m] A *thy*⁺ derivative of B840c produced by transduction.

[n] A *pyrA*⁻ derivative of 310 produced by selecting a *leu*⁺ *pyrA*⁻ recombinant from the following transduction: donor 311 (*thr*⁻ *pyrA*⁻ *ara*⁻) × recipient 310 (*deoC*⁻ *ara*⁻ *leu*⁻).

[o] A *thy*⁺ derivative of 303 produced by transduction.

[p] Obtained as a *thr*⁺ *deoA*⁻ recombinant from the following cross: K12SH-28 (*thr*⁺ *deoA*⁻) (12) × 297 (*thr*⁻ *deoA*⁺).

[q] Obtained from D. Sheppard (32).

studies were carried out in an atmospheric medium. The system was allowed to reach 37 C, and the nucleoside or water was tipped into the culture. In some cases (not presented), other compounds such as uridine were added from a second arm after determining the initial rate of oxygen uptake in the presence of the deoxyribonucleoside inhibitor. The details are given in the legends of the figures.

Preparation of extracts. Cultures were harvested by centrifugation at 6,000 × *g* for 15 min in the cold. The cells were washed once with minimal medium and recentrifuged. Extracts were prepared by grinding cells with alumina as described previously (31) and extracting with 0.05 M tris(hydroxymethyl)aminomethane-chloride, *p*H 7.4, containing 10^{-2} M 2-mercaptoethanol and 10^{-3} M ethylenediaminetetraacetate.

Enzyme assays. TdR phosphorylase was assayed by the method of Friedkin and Roberts (13). dR-5-P aldolase was measured by the procedure of Racker (29), with the addition only of alcohol dehydrogenase (Worthington Biochemicals Corp., Freehold, N. J.) and reduced nicotinamide adenine dinucleotide (Sigma Chemical Co., St. Louis, Mo.) to the reaction mixture in order to follow acetaldehyde formation, or by measurement of dR-5-P formation (29).

Chemical analysis of cultures for deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein. The acid-insoluble material from 10 ml of culture was precipitated by the addition of 1 ml of cold 55% perchloric acid (PCA), separated by centrifugation, washed twice with cold 5% PCA, and resuspended in 1.25 ml of 5% PCA. The nucleic acids were extracted by heating for 20 min at 90 C. After centrifugation, constant-size samples of supernatant fluid were analyzed for DNA by the Burton modification of the diphenylamine reaction (9) with deoxyadenosine as the standard, and for RNA by the orcinol method (1) with adenosine as the standard. The residue was dissolved in 5 ml of 0.02 M NaOH-0.02 M Na_2CO_3, and was analyzed for protein by the procedure of Lowry et al. (24).

Analysis of culture filtrates for thymine and deoxyribose. Portions of cultures were filtered through HA45 filters (Millipore). The filtrate was analyzed for deoxyribose by the diphenylamine reaction. TdR does not react in the diphenylamine test. Thymine was determined from the absorbance of samples in 0.1 N NaOH at 300 mμ (19).

Chemicals. TdR (A grade) and dR-5-P were obtained from Calbiochem, Los Angeles, Calif. All other deoxyribonucleosides, ribonucleosides, and bases used in this study were obtained from commercial sources, primarily Calbiochem. Trimethoprim was the generous gift of G. H. Hitchings, Burroughs-Wellcome Laboratories. DL-Glyceraldehyde 3-phosphate was prepared from the diethylacetal barium derivative from Calbiochem. The D-glyceraldehyde 3-phosphate concentration was measured by coupling to α-glycerophosphate dehydrogenase and triosephosphate isomerase (Calbiochem).

RESULTS

Identification of deoB and deoC mutants. The *deoB* and *deoC* mutants were discovered when several low thymine-requiring *thy* mutants were tested for their relative rates of growth on minimal agar plates supplemented with thymine, TdR, or dTMP, by measuring the average size of the colonies. Two of these strains displayed unexpected growth characteristics on TdR-agar plates, as shown in Table 2. All mutants formed small but distinguishable colonies on thymine-agar within 24 hr. Neither B840c nor B/r-62 formed colonies on thymidine-agar within 24 hr. The appearance of visible colonies after 48 hr indicated that the effect of thymidine was not bactericidal. All strains appeared to grow more slowly on dTMP at the level employed.

TABLE 2. *Average size of colonies produced by thy mutants on minimal agar supplemented with thymine, thymidine, or thymidylate*[a]

Strain	Thymine (10 μg/ml)		Thymidine (20 μg/ml)		Thymidylate (30 μg/ml)	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
B3 (71).....	1.1[b]	3.3	0.8	3.8	1.1	4.1
B307c.....	2.1	5.6	1.9	4.7	1.2	1.8
B840c.....	1.7	4.5	0	0.9	1.0	1.0
B/r-62.:....	1.4	3.5	0	1.8	0.7	1.3

[a] The diameter of individual colonies was measured under a low-power Bausch & Lomb stereoscopic microscope fitted with an ocular rule. The values given represent the average of 10 measurements and are in mm.

Further studies on the growth characteristics of these mutants in liquid medium supplemented with either thymine (10 μg/ml) or TdR (20 μg/ml) showed that in TdR B/r-62 and B840c grew at a greatly reduced rate for 2 hr, after which growth abruptly resumed at the normal rate. The growth of B307c was normal in either medium. Therefore, low thymine-requiring strains, such as B307c, which grow normally on either thymine or TdR were designated *deoB* mutants, and strains such as B840c and B/r-62 which could grow on low levels of thymine but were inhibited by TdR were called *deoC* mutants. The complete phenotypes of these two classes of mutants are summarized in Table 3.

Expression of deoC mutation in thy$^+$ and thy$^-$ backgrounds. Both B840c and B/r-62 were converted to *thy*$^+$ derivatives by transduction with phage P1bt which had previously been grown on a wild-type organism. All *thy*$^+$ transductants still grew slowly on TdR, indicating that this effect was an inhibition unrelated to the nutritional requirement imposed upon the cell by the *thy* mutation. Furthermore, the *deoC* mutation was not linked to the *thy* locus by transduction. These results are summarized in Table 4, which lists the growth rate as mass doubling time of the original strains and *thy*$^+$ transductants in medium supplemented with thymine or high concentrations of TdR. It can also be seen from these data that TdR had no effect on the growth rate of wild-type or *deoB*$^-$ *thy*$^-$ cells.

TABLE 3. *Phenotypes of deoB and deoC mutants*

Phenotype	Genotype			
	deoB$^+$ *deoC*$^+$ *thy*$^-$	*deoB*$^-$ *deoC*$^+$ *thy*$^-$	*deoB*$^+$ *deoC*$^-$ *thy*$^-$	*thy*$^+$ *deoB*$^+$ *deoC*$^-$
Can utilize 1 to 2 μg of thymine per ml for growth	No	Yes	Yes	Not testable
Can utilize thymidine as a sole carbon source	Yes	No	No	No
Growth inhibited by deoxyribonucleosides	No	No	Yes	Yes
Thymidine phosphorylase activity	Inducible	Uninducible	Inducible	Inducible
Deoxyribose 5-phosphate aldolase	Inducible	Uninducible	Absent	Absent

Inhibition of growth of a deoC mutant by TdR. The kinetics of growth of a *thy*$^+$ *deoC*$^-$ strain in cultures containing identical initial cell densities but different amounts of thymidine was investigated. The length of the lag period was directly proportional to the concentration of TdR in the medium (Fig. 1). If 50 μg of TdR per ml was added to a sample of the same culture at a lower initial cell density, the length of the lag period was increased proportionately to the decrease in cell density. Thus, the length of the inhibitory phase was a function of the concentration of TdR per cell.

This proportionality and the fact that inhibition was reversible suggested that the effect might be due either to the TdR itself or to a closely related metabolite whose intracellular concentration could be maintained at an elevated level so long as a sufficiently high concentration of TdR remained in the medium. Therefore, the fate of the added TdR and the relationship between TdR degradation and inhibition were examined by measuring the appearance of thymine and deoxyribose in the medium. As shown in Fig. 2, the time required for complete conversion of TdR to thymine and deoxyribose coincides with the length of time the culture remained inhibited. The thymine and deoxyribose found in the culture filtrate represented 100% and 75%, respectively, of the TdR originally present, a finding not greatly different from the results with *E. coli* 15 *thy*$^-$ (70V3) (5) in which equimolar concentrations of thymine and deoxyribose were found. The deoxyribose could be either free deoxyribose or a phosphorylated derivative, since the assay for deoxyribose would not distinguish between these compounds.

Inhibition by TdR of the synthesis of nucleic acids and protein in a deoC mutant. To determine which metabolic process was being inhibited by TdR and thus limiting the rate of growth, we investigated the kinetics of the synthesis of nucleic acids and protein. The results shown in Fig. 3 indicate that, when TdR was added to an exponentially growing culture of *thy*$^+$ *deoC*$^-$ cells, protein and RNA synthesis were quickly inhibited. DNA synthesis appeared to be inhibited only after a delay of 15 or 20 min. Since these results might derive from limitation of an energy supply, the effect of thymidine on the respiration of *deoC*$^-$ cells was investigated.

TABLE 4. *Summary of growth rates of deoB and deoC mutants in minimal medium supplemented with either thymine or thymidine*[a]

Genotype	Strain	Mass doubling time (min)		
		Minimal	Thymine, 10 μg/ml	Thymidine, 200–500 μg/ml
deoB$^+$ *deoC*$^+$ *thy*$^+$	B	43	49	44
deoB$^-$ *deoC*$^+$ *thy*$^-$	B307c	—	49	47
	302	—	48	48
deoB$^+$ *deoC*$^-$ *thy*$^-$	B840c	—	49	127–267
	B/r-62	—	67	159–192
	303	—	31[b]	100–116[b]
deoB$^+$ *deoC*$^-$ *thy*$^+$	315	45	45	164
	296	43	—	140

[a] The mass doubling time for *deoC* mutants growing in thymidine was determined from the inhibitory phase of the growth cycle. The value for *deoC* mutants in minimal medium or minimal medium plus thymine and for *deoC*$^+$ strains under all conditions were determined from the exponential phase of the growth cycle. The range of values given for the mass doubling time of *deoC* mutants represents the values obtained in several different experiments.

[b] The medium was supplemented with vitamin-free acid-hydrolyzed casein (0.2%) and L-tryptophan (20 μg/ml) in addition to thymine or thymidine.

Inhibition by TdR of oxygen uptake. The effect of TdR on the rate of oxygen uptake by suspensions of *thy*$^+$ *deoC*$^-$ cells in a glucose-salts medium can be seen in Fig. 4. In this experiment, the cul-

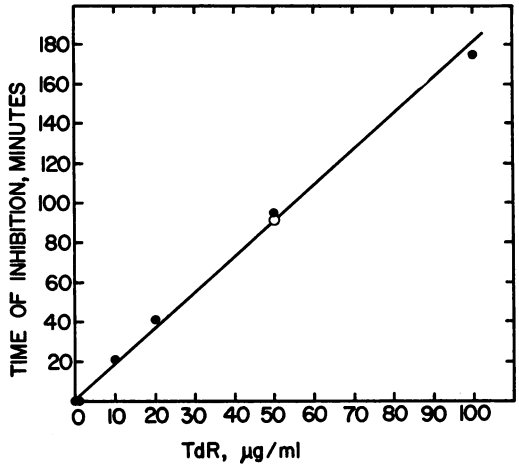

FIG. 1. *Influence of thymidine concentration on the length of the period of inhibition of a deoC mutant. Thymidine was added to samples of strain 315 (deoC⁻ thy⁺) growing exponentially in minimal medium. Growth was followed turbidimetrically until each culture had overcome the inhibition by thymidine and had reached late stationary phase. The length of the period of inhibition was calculated from the resulting growth curves by extrapolating the two linear portions of a semilog plot. The solid circles represent data from cultures to which the thymidine had been added when the cell density was 20 Klett colorimeter units. One culture which received thymidine (50 μg/ml) at a Klett reading of 8 had a period of inhibition of 229 min. When this value was corrected for the difference in the initial cell density of the culture, the value (○) also fell on the line.*

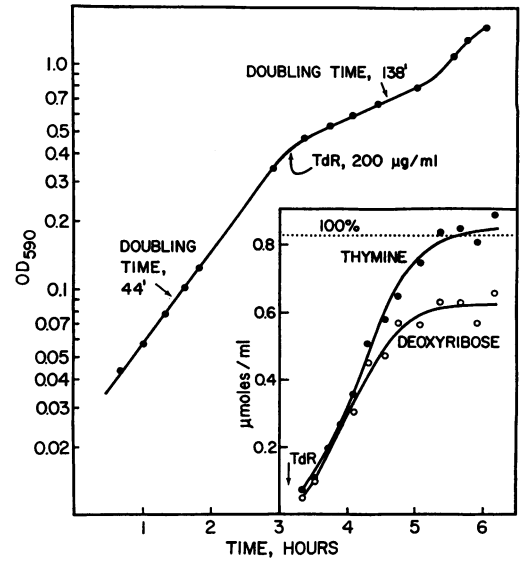

FIG. 2. *Kinetics of the appearance of thymine and deoxyribose in the culture medium of a deoC mutant inhibited by thymidine. Thymidine was added at the indicated time to an exponentially growing culture of strain 296 (thy⁺ deoC⁻) to yield a final concentration of 200 μg/ml (0.83 μmole/ml).*

tures were allowed to come to equilibrium in the Warburg flasks, and at time-zero the indicated quantities of TdR were tipped into the main compartment of the flask and oxygen uptake was measured. Because the cells had been exposed to low temperatures, there was a short lag period before a linear rate of oxygen uptake was obtained with the control vessel containing no TdR. Increasing the concentration of TdR in the reaction vessel greatly increased this lag period. TdR added during oxygen-uptake measurements promptly caused inhibition. Figure 5 shows that TdR had no effect upon the rate of oxygen uptake by the wild-type strain (B), whereas the *thy*⁺ *deoC*⁻ strain (296) was severely inhibited. Uridine was able to overcome partially the inhibitory effect of TdR. This effect also was observed when uridine was added after the TdR inhibition had developed. By means of the disc-diffusion technique, it was determined that TdR showed the same inhibition of growth of strain 296 when glucose, acetate, or succinate was employed as the carbon source. That the decreased oxygen uptake is a result of an inhibition of respiration, and not due to a difference in the mass of cells, is shown by the following. (i) An immediate decrease occurs in oxygen uptake of about two to five times below the control value in the presence of TdR. In other experiments (not shown), TdR was added while the oxygen uptake was being measured, and an immediate decrease in uptake occurred. (ii) The growth rate in these small vessels with the beginning concentration of 4×10^8 cells/ml and with minimal agitation was quite slow, with doubling times of perhaps 80 min.

Inhibition of deoC strains by other deoxyribonucleosides. By means of the disc diffusion assay, several bases, ribonucleosides, and deoxyribonucleosides were tested in place of TdR as possible inhibitors of *deoC*⁻ strains. The results shown in Table 5 indicate that all deoxyribonucleosides tested, except deoxycytidine, inhibited this strain. On the basis of isotope dilution experiments, Lichtenstein et al. (23) have suggested that deoxycytidine is metabolized differently than TdR. Ribonucleosides did not inhibit *thy*⁺ *deoC*⁻ strains. The free bases had no effect on any strain tested, and none of the compounds inhibited the wild-type control. However, if 0.5 mg of any ribonucleoside was added to the top-layer agar and TdR was placed on the disc, either no zone of inhibition was observed or the zone of inhibition was greatly decreased.

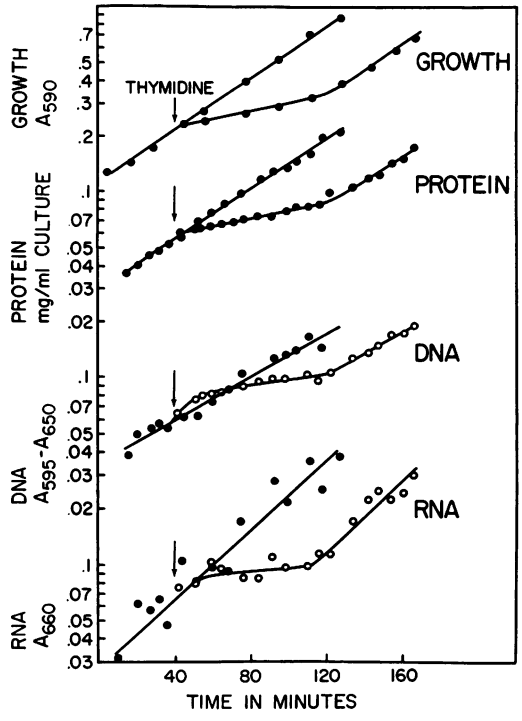

FIG. 3. *Kinetics of growth, protein synthesis, DNA synthesis, and RNA synthesis of a deoC mutant inhibited by thymidine. Thymidine (50 μg/ml) was added to one-half of a culture of strain 296 (thy⁺ deoC⁻) growing exponentially in minimal medium at the time indicated by the arrow. Samples of the inhibited culture and the control culture containing no thymidine were assayed for protein, DNA, and RNA. An A_{595}–A_{650} reading of 3.83 is equivalent to 1 μmole of deoxyadenosine in the diphenylamine reaction. An A_{660} of 14.5 is equivalent to 1 μmole of adenosine in the orcinol reaction.*

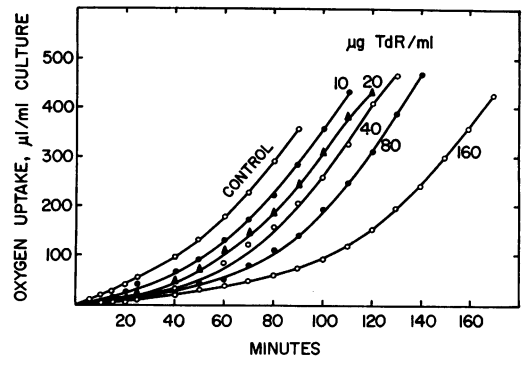

FIG. 4. *Inhibition of the oxygen uptake of a deoC mutant with increasing concentrations of thymidine. Cultures of E. coli 296 (deoC⁻) were grown aerobically to a cell concentration of 4 × 10⁸/ml; 1-ml portions were added to each flask. After equilibration at 37 C, 0.1 ml of TdR or water was tipped in from the side arm, and measurements were made.*

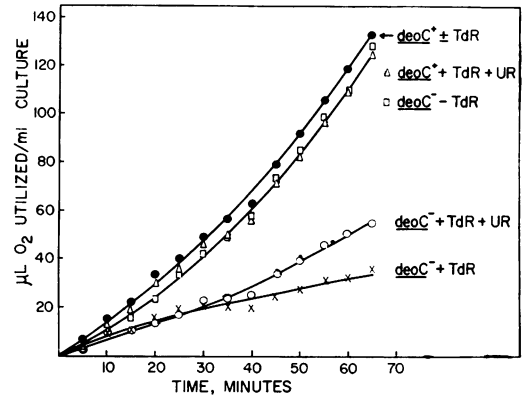

FIG. 5. *Inhibition by thymidine of the oxygen uptake of deoC⁻ strains and insensitivity of deoC⁺ strains. Cultures of E. coli 296 (deoC⁻) and E. coli B (deoC⁺) were grown and treated as in the legend to Fig. 4. TdR (200 μg) and uridine (200 μg) were added to the indicated vessels at zero-time. The final volume was 1.2 ml.*

Mapping of the deoB and deoC genes. In conjugation studies involving a prototrophic Hfr and a *thy⁻ deoB⁻ thr⁻* female (strain 302), we observed that 91% of the *thr⁺* recombinants had received the *deoB⁺* marker. The linkage of *deoB* to *thr* was confirmed by transductional crosses, which yielded a cotransduction frequency of 48%. The *deoC* locus is also linked to *thr*. The results of transduction experiments in which two different *deoC* mutants were used as donors indicated that the *deoC* marker was transferred with the *thr⁺* marker at a frequency of about 50% (Table 6). Therefore, the sensitivity to deoxyribonucleosides was not the result of several unlinked mutations in the original strains.

Three-factor crosses to order the *deoC* gene with respect to *thr* and *pyrA* were also performed with the three different *deoC⁻* alleles in our collection. The results shown in Table 7 suggest that the most probable order of markers is: *deoC thr pyrA*.

The results of a three-factor cross to order the *deoA*, *deoC*, and *thr* markers are presented in Table 8. The low frequency of recombination between the *deoA* and *deoC* markers indicates that they are very closely linked. In addition the absence of *deoA⁻ deoC⁻* recombinants and the presence of only a few *deoA⁺ deoC⁺* recombinants is taken to indicate that the *deoA⁻ deoC⁻* class requires four crossing-over events. There-

fore, the most probable order of markers is: *deoA deoC thr*. Figure 6 shows the position of these genes in relationship to other markers on the chromosome of *E. coli*.

Dominance of the deoC⁺ and deoB⁺ alleles.

TABLE 5. *Effect of deoxyribonucleosides and related compounds on a deoC mutant (296)*[a]

Compound tested[b]	Concn	Zone of inhibition
	mg/ml	*mm*
Thymidine	10	17
Deoxyuridine	10	17.5
Deoxycytidine	10	1
Deoxyadenosine	5	14.5
Deoxyguanosine	10	17
Bromodeoxyuridine	10	12.5

[a] Inhibition was measured by the disc diffusion assay. The concentration given is of the test solution applied to the disc. The plates were incubated for 24 hr.

[b] The following bases were not inhibitory at 1 mg/ml: thymine, uracil, adenine, cytosine, and guanine. Adenosine (5 mg/ml) and the following ribonucleosides (10 mg/ml) were not inhibitory: thymine riboside, uridine, cytidine, and guanosine (saturated solution). Deoxyribose (10 mg/ml) was not inhibitory. None of the compounds tested inhibited the wild-type control.

Merodiploids heterozygous for the *deoC* and *deoB* markers were constructed by the introduction of an episome (F′ *ara*) which carries the wild-type alleles of both markers. This episome arose from Hfr B2, which has a point of origin near *thr* and transfers markers in a counter-clockwise direction (32). The merodiploid with the genotype *deoB⁺ deoC⁻ thy⁻* (strain 318)/F′ *araB24 deoC⁺* was resistant to deoxyribonucleosides and required high levels of thymine for growth. The merodiploid having the genotype *deoB⁻ deoC⁺ thy⁻* (strain 302)/F′ *araB24 deoB⁺ deoC⁺* required high levels of thymine for growth. These results demonstrate that *deoB⁺* and *deoC⁺* are dominant to their mutant alleles and that these genes must normally be responsible for the formation of physiologically active products.

dR-5-P aldolase and TdR phosphorylase activities in deoB and deoC mutants with and without induction by TdR. Extracts of various strains grown in the presence or absence of levels of TdR sufficient to induce TdR phosphorylase (100 μg/ml or greater) (30) were assayed for dR-5-P aldolase and for TdR phosphorylase activities (Table 9). The results indicate that TdR induces the formation of both the aldolase and TdR phosphorylase in the wild-type strain (*E. coli* B). However, in *deoB* mutants, induction of phosphorylase and aldolase did not occur or

TABLE 6. *Cotransduction of deoC and thr markers*[a]

Donor	Genotype of donor	Genotype of recipient	No. of *thr⁺* recombinants tested	No. *deoC⁻*	Cotransduction frequency (%)
B/r-62	*thr⁺ deoC⁻*	*thr⁻ deoC⁺*	104	46	44
B840c	*thr⁺ deoC⁻*	*thr⁻ deoC⁺*	104	55	53

[a] P1 lysate made on the indicated donor was used to infect the recipient strain 297. The *deoC* marker was tested by spotting suspensions of single colonies on plates containing TdR (200 μg/ml).

TABLE 7. *Ordering of deoC, thr, and pyrA by a three-factor transductional cross*[a]

Classes of recombinants	Cross I[b]: *deoC⁻ thr⁺ pyrA⁺ → deoC⁺ thr⁻ pyrA⁻*			Cross II[c]: *deoC⁺ thr⁺ pyrA⁺ → deoC⁻ thr⁻ pyrA⁻*	
	No. of recombinants if donor is:		Required crossing-over regions[d]	No. of recombinants	Required crossing-over regions[d]
	B/r-62	B840c			
pyrA⁺ thr⁺ deoC⁻	16	12	1–4	28	2–4
thr⁺ deoC⁺	19	15	2–4	19	1–4
thr⁻ deoC⁻	0	0	1–2–3–4	41	3–4
thr⁻ deoC⁺	19	27	3–4	2	1–2–3–4

[a] The *pyrA⁺* recombinants were purified on selective media and tested for *thr* and *deoC* markers.

[b] The recipient for this cross was strain 300.

[c] The donor in this cross was strain 104 and the recipient strain 322.

[d] Crossing-over regions (as shown in parentheses) if the order of markers is: (1) – *deoC* – (2) – *thr* – (3) – *pyrA* – (4).

TABLE 8. *Ordering of deoA, deoC, and thr by a three-factor transductional cross*[a]

Class of recombinants	No. in class	Required crossing-over regions[b]
thr$^+$ *deoA*$^+$ *deoC*$^-$	37	3–4
thr$^+$ *deoA*$^-$ *deoC*$^+$	45	1–4
thr$^+$ *deoA*$^+$ *deoC*$^+$	2	2–4
thr$^+$ *deoA*$^-$ *deoC*$^-$	0	1–2–3–4

[a] P1 lysate prepared on the donor strain 299 (*deoA*$^-$ *deoC*$^+$ *thr*$^+$) was used to infect the recipient strain 339 (*deoA*$^+$ *deoC*$^-$ *thr*$^-$). The *thr*$^+$ recombinants (84) were selected and purified on selective agar. The *deoA* and *deoC* markers were tested in the following manner. The *deoA*$^+$ *deoC*$^+$ recombinants were able to grow on thymidine as sole carbon source. Neither *deoA*$^-$ nor *deoC*$^-$ mutants would grow on this medium. The *deoA*$^+$ *deoC*$^-$ recombinants were inhibited when streaked on minimal-glucose agar containing a high concentration of TdR (200 μg/ml). Recombinants that did not grow on TdR as sole carbon source but were not inhibited by TdR were assumed to be *deoA*$^-$ recombinants; however, this class would include both *deoA*$^-$ *deoC*$^+$ and *deoA*$^-$ *deoC*$^-$ recombinants. The *deoA*$^-$ *deoC*$^-$ recombinants should be inhibited by purine deoxyribosides such as AdR. The *deoA*$^-$ class was tested for AdR inhibition by the disc-diffusion assay; no AdR-sensitive recombinants were found. The *deoA* marker was independently confirmed by determining whether cultures of each recombinant could degrade TdR to thymine and deoxyribose.

[b] Crossing-over regions (shown in parentheses) if the order is: (1) – *deoA* – (2) – *deoC* – (3) – *thr* – (4).

was only minimal. On the other hand, TdR phosphorylase was slightly elevated in *deoC*$^-$ cells and was greatly increased after exposure of the culture to TdR. In sharp contrast, TdR-induced or uninduced *deoC*$^-$ cells showed negligible or very low levels of dR-5-P aldolase. In some instances, a slight and anomalous activity appeared to be present immediately after addition of the dR-5-P substrate to extracts of *deoC*$^-$ cells but stopped within minutes, whereas the activities observed in *E. coli* B and in *deoB*$^-$ cells continued linearly for periods of over 1 hr.

Strain 388 carrying a mutation in the *deoA* gene and lacking TdR phosphorylase (12) showed induced levels of dR-5-P aldolase when grown on deoxyadenosine. Strain 71 was obtained from *E. coli* B3 (our original culture obtained from N. Melechen in 1959) as a single-colony isolate on plates containing 2 μg of thymine/ml. Strain 71 had constitutive levels of both TdR phosphorylase and dR-5-P aldolase, about 20 times greater than the uninduced wild-type levels. These activities

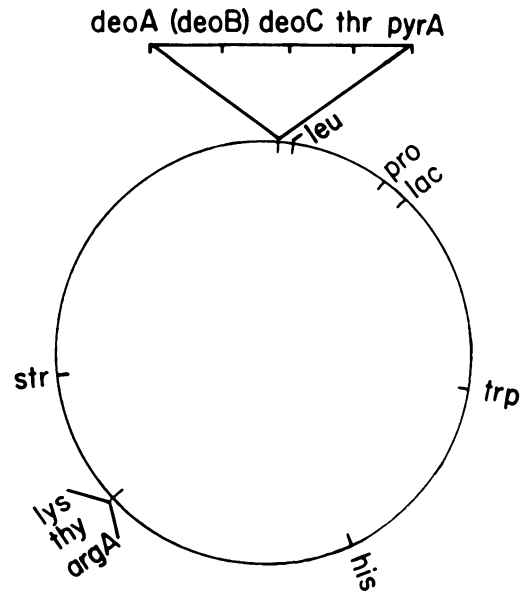

FIG. 6. *Genetic map (35) of E. coli showing the position of the deo genes.*

could be induced slightly further by growth in TdR. A culture of *E. coli* B3 (strain 72) obtained from Dr. Melechen in 1965 had approximately normal levels of both dR-5-P aldolase and TdR phosphorylase activities, but these were increased only minimally by exposure to TdR. Neither strain 71 nor strain 72 is inhibited by TdR, and neither grows on TdR as the sole carbon source; both are therefore classified as *deoB*$^-$ organisms. The TdR phosphorylase to dR-5-P aldolase ratios appear to be increased in strain 71, but in both strain 72 (ratios not shown) and strain 71 the ratios were essentially the same in induced and uninduced cultures.

Because some extracts exhibited a relatively rapid oxidation of reduced nicotinamide adenine dinucleotide, it was frequently not possible to assay dR-5-P aldolase by a one-step direct coupling of the acetaldehyde to the alcohol dehydrogenase reaction (or D-glyceraldehyde 3-phosphate to triose isomerase and glycerol-phosphate dehydrogenase), especially when the aldolase level was low. Accordingly, in some instances aldolase activity was assayed by measuring the rate of formation of dR-5-P from acetaldehyde and glyceraldehyde 3-phosphate (29) at 25 C, by use of the Burton (9) modification of the diphenylamine reaction. By this procedure, very low levels of aldolase activity could be detected without background interference. These results are shown in parentheses in Table 9.

TABLE 9. *Levels of dR-5-P aldolase and TdR phosphorylase in extracts of deoA, deoB, and deoC mutants*[a]

Genotype	Strain	dR-5-P aldolase (units/mg of protein)[b]		TdR phosphorylase (units/mg of protein)[c]		Phosphorylase/aldolase	
		Uninduced	Induced	Uninduced	Induced	Uninduced	Induced
Wild type	B	6.7–7.2	87–127	92–95	1,240–1,250	13.4[d]	11.9[d]
	B	(2.8)[e]	(43)[e]				
deoB⁻ thy⁻	B307c	7.9	15.3	77	170	9.7	11.1
	B307c	(13.8)	(10.0)				
	201	13.3	11.5	128	115	9.6	10.0
	72(B3)	(6.3)	(8.4)	114	172		
	71(B3)	102	134	1,470	2,130	14.5	15.9
deoC⁻ thy⁻	B840c	0[f]	0[f]	177	2,220		
	B/r-62	<1.5			720		
deoC⁻ thy⁺	296	0[f]	0[f]	135	1,180		
	296	(0)	(0)				
deoA⁻ thy⁺	388		97		6.7		

[a] Uninduced values are from cultures grown in minimal medium (*thy⁺* strains) or 10 μg/ml of thymine (*thy⁻* strains). Induced values are from cultures grown in 500 μg/ml of thymidine, except for strain 388 in which 500 μg/ml of AdR was used as the inducer.

[b] Product formed (mμmoles) per minute at 25 C.

[c] Product formed (mμmoles) per minute at 37 C.

[d] When more than one enzyme value is presented, extracts of at least two separate cultures were assayed, and the ratios are based on average values.

[e] The figures in parentheses represent the rates of formation of dR-5-P (9) from acetaldehyde and D-glyceraldehyde 3-phosphate (29). The two values in parentheses opposite strains B307c and B are from different extracts than those assayed by the forward reaction.

[f] Anomalous rate first few minutes; *see* Results.

DISCUSSION

These studies provide evidence that: (i) mutations in either of two genes, *deoB* or *deoC*, will explain the mutation of a *thy⁻* organism from a high to a low thymine requirement; (ii) the inhibition of the growth of *deoC* mutants by deoxyribonucleosides appears to result from an inhibition of respiration; (iii) the *deoC* gene determines dR-5-P aldolase; and (iv) dR-5-P aldolase and TdR phosphorylase (determined by the *deoA* gene) are coordinately induced by thymidine and are controlled by genes in an operon responsible for the degradation of TdR. To account for these findings, we present a model which proposes that *deoB* determines phosphodeoxyribomutase and that dR-5-P is both the inducer of this operon and the inhibitor of *deoC* mutants. The phenotypes of *deoB* and *deoC* mutants are summarized in Table 3.

Figure 7 describes the pathway for the degradation of TdR (and other deoxyribonucleosides) and the gene-enzyme relationships suggested by the present studies. The following evidence has been presented to support these gene assignments.

The *deoC* mutants clearly lack dR-5-P aldolase activity (Table 9). In such mutants, TdR phosphorylase is present at wild-type levels and is inducible by TdR. These *deoC* mutations are therefore not in a regulatory gene affecting all the enzymes of the pathway. This conclusion is also supported by the observation that the wild-type allele is dominant in merodiploids.

Because *deoB* mutants cannot grow on TdR as a sole source of carbon, they must be blocked in some step in the conversion of TdR to a source of energy. Since *E. coli* is unable to utilize either thymine or free deoxyribose as an energy source, the energy is considered to be derived from a deoxyribose phosphate moiety. Extracts of *deoB* mutants have been shown (Table 9) to contain wild-type levels of TdR phosphorylase and dR-5-P aldolase, two of the three enzymes required for the conversion of TdR to compounds which are intermediates in an energy-yielding pathway. Therefore, we conclude that the *deoB* mutants lack the third enzyme, phosphodeoxyribomutase, although this deduction has not yet been verified directly by enzyme assays. The fact that the *deoB⁺* allele is also dominant to the *deoB⁻* allele in merodiploids supports the conclusion that *deoB* mutants are deficient in some enzymatic function and are not regulatory mutants of the O° type, although the dominance relationship per se does not eliminate a mutation of a regulatory gene of the positive control type (32).

These gene-enzyme relationships also provide a model to explain the phenotypes of *deoB* and *deoC* mutants. Loss of either phosphodeoxyribomutase (*deoB*) or dR-5-P aldolase (*deoC*) would cause an accumulation of dR-1-P and an enhancement of the ability of a *thy*$^-$ cell to utilize low concentrations of thymine for DNA synthesis. However, mutants of these two genes would be expected to differ with respect to other properties because different deoxyribose phosphates would accumulate in each case. Only dR-1-P should accumulate in *deoB* mutants, whereas both dR-1-P and dR-5-P should accumulate in *deoC* mutants. Assuming that dR-5-P is the inhibitor (or the precursor of the inhibitor) of *deoC* mutants, it is to be expected that *deoB*$^-$ cells, which cannot form dR-5-P, would be insensitive to deoyxribonucleosides.

This model also is consistent with the results of studies of the induction of thymidine phosphorylase in *deoC* and *deoB* mutants. In the wild-type organism, this activity can be increased more than 10-fold by the addition of high concentrations of TdR to the growth medium. The observation that all deoxyribonucleosides induce TdR phosphorylase activity in *E. coli* K-12 had led to the suggestion that dR-1-P could be the inducer (30). On the basis of our findings, summarized in the model shown in Fig. 7, the inducer of both TdR phosphorylase and dR-5-P aldolase is considered to be dR-5-P. TdR phosphorylase and dR-5-P aldolase activities could not be induced or showed only minimal induction in *deoB* mutants (Table 9), which according to our model cannot form dR-5-P. On the other hand, TdR phosphorylase was induced in *deoC* mutants, which are able to form dR-5-P, and was normally higher in the absence of added inducer than the levels found in uninduced wild-type cells. The different patterns of induction of TdR phosphorylase observed in *deoB*$^-$ *thy*$^-$ and *deoC*$^-$ *thy*$^-$ strains help to explain the apparently contradictory reports of other workers concerning the inducibility or noninducibility of this enzyme in low thymine-requiring *thy*$^-$ mutants (5, 6, 16, 30) and emphasizes the importance of distinguishing between these two classes of mutants when attempting to describe the properties of *thy*$^-$ strains.

Breitman and Bradford (5) also had suggested that the inducer could be dR-5-P. In a very recent report, these workers (7) have found that certain low thymine-requiring mutants of *E. coli* 15 *thy*$^-$ lacked phosphodeoxyribomutase activity and, therefore, would not be expected to form dR-5-P. TdR phosphorylase and dR-5-P aldolase could not be induced in these mutants, although in the parental strain these enzymes were apparently coordinately induced (5, 7). Their findings

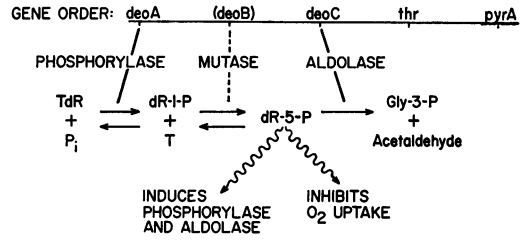

FIG. 7. *The deo gene-enzyme complex of E. coli. The deoB gene has not been ordered by genetic tests but has arbitrarily been placed between deoA and deoC on the basis of the sequence of enzymatic reactions. Abbreviations used: phosphorylase, TdR phosphorylase; mutase, phosphodeoxyribomutase; aldolase, deoxyribose 5-phosphate aldolase; P_i, orthophosphate; dR-1-P, deoxyribose 1-phosphate; dR-5-P, deoxyribose 5-phosphate; gly-3-P, glyceraldehyde 3-phosphate.*

strengthen the model presented in this paper and our assignment of the mutase activity to the *deoB* gene in *E. coli* B.

Recent studies by Hoffee (17) on deoxyribose-negative mutants of *Salmonella typhimurium* also are consistent with the hypothesis that dR-5-P is the inducer of the thymidine pathway in *E. coli*. *S. typhimurium* can utilize free deoxyribose as the sole carbon source by virtue of an inducible pathway consisting of a permease, deoxyribose kinase, and a dR-5-P aldolase, all of which are coordinately induced by deoxyribose. However, this organism contains a second form of dR-5-P aldolase which is induced by dR-5-P and coordinately regulated with TdR phosphorylase. Mutants of this second form of dR-5-P aldolase map in the *thr-ara* region of the *S. typhimurium* genetic map and are therefore analogous to our *deoC* mutants (P. Hoffee, *personal communication*).

The close linkage of the *deo* genes (Tables 6–8) and the clear biochemical relationship of their products suggest that these genes may constitute an operon whose enzyme products are required for the degradation of TdR and deoxyribose-phosphates. *DeoA* and *deoC* are clearly very closely linked (Table 8). Although the *deoB* gene has not been ordered in these studies, it shows the same frequency of cotransduction with *thr* as do *deoA* and *deoC* (about 50%). The mapping data of other workers (2, 25) places all low-thymine mutations to the left of *thr*. We have therefore arbitrarily placed the *deoB* gene between *deoA* and *deoC*.

The concept of a *deo* operon is further supported by our observations that dR-5-P aldolase and TdR phosphorylase are coordinately induced by TdR (Table 9). Other workers have shown that TdR phosphorylase (5, 28, 30),

phosphodeoxyribomutase (7; H. O. Kammen, Federation Proc., p. 809, 1967), and dR-5-P aldolase (5, 7) were inducible by TdR. The requirement that an operon be regulated is also met by the finding that strain 71, classified as *deoB*$^-$, and derived from *E. coli* B3, is constitutive for both TdR phosphorylase and dR-5-P aldolase and thus may represent a regulatory mutation of the O^c type. The exact nature or location of the mutation in this organism remains to be determined and is presently under investigation.

The mutation to the low thymine requirement has been described by Breitman and Bradford (4), Stacey and Simson (34), and Harrison (16), and has recently been mapped by Alikhanian et al. (2) and by Okada (25). The mutation has been called *ltr* and *thyR*, respectively, by the latter two groups. Alikhanian and co-workers (2) also described a mutation to TdR sensitivity (*td*^{s}), but suggested that the low thymine requirement of the same *thy*$^-$ strain might be due to the presence of an additional mutation in the *ltr* gene. Our work confirms and extends the genetic studies of these groups on mutations leading to a low thymine requirement (*deoB* or *deoC*) and to sensitivity to deoxyribonucleosides (*deoC*). In addition, the data presented here establish that *deoC* mutants show the low thymine phenotype as well as deoxyribonucleoside sensitivity as a consequence of the loss of dR-5-P aldolase activity. These observations are in accord with the findings of Breitman and Bradford (5) that a low thymine-requiring *thy*$^-$ strain of *E. coli* 15 (70V3) lacked dR-5-P aldolase. These workers also had observed that the growth of this strain was inhibited by thymidine (5, 6) but did not relate the inhibition to the loss of dR-5-P aldolase. In fact, they report that thymidine inhibited the growth of *E. coli* 15 *thy*$^-$ and *E. coli* 15, I, and II, all of which lacked phosphodeoxyribomutase activity (7). At the present time, we have no explanation for the differences between the results of these authors and those described here. Whether these are species differences or other kinds of mutations will have to await further study. The simultaneous genetic, enzymatic, and physiological studies reported in the present work clearly establish that the inhibition, low thymine requirement, and loss of dR-5-P aldolase are phenotypic expressions of the same mutation in *E. coli* B. Eisenstark and co-workers (*personal communication*) have found that *S. typhimurium* shows a mutation to deoxyribonucleoside sensitivity and that the same gene is responsible for a low thymine requirement in *thy*$^-$ cells. Their mutants appear to correspond to our *deoC* mutants and to the mutants of Hoffee determining the second form of dR-5-P aldolase in *S. typhimurium* (17).

The inhibitory effect of deoxyribonucleosides on the oxygen uptake of *deoC*$^-$ cells (Fig. 4 and 5) is considered to be on the respiratory chain rather than in the carbon pathway, inasmuch as growth was inhibited about equally well with glucose, succinate, or acetate as the carbon source. Since all macromolecular syntheses tested (DNA, RNA, and protein) were found to be inhibited by TdR, it has been concluded that the inhibition occurs by a limitation in the energy source. The authors are aware of no evidence that inhibition of protein, RNA, or DNA synthesis will decrease respiration. Accordingly, the effect on respiration is considered to be the primary action of TdR. The site of action and the mechanism by which the apparent agent, dR-5-P, effects this inhibition remain to be studied. The data in Fig. 5 and the text indicate that ribonucleosides could overcome the inhibitory effects of TdR. This effect of ribonucleosides may be explained by the recent demonstration that these compounds inhibit TdR phosphorylase (8) and thus may act by preventing the formation of dR-5-P.

Although the method of analysis of the deoxyribose excreted into the medium when TdR was added to *deoC*$^-$ cells did not distinguish between deoxyribose and its phosphorylated derivatives, in all probability free deoxyribose was excreted. Breitman and Bradford (4) have shown that free deoxyribose was excreted by a low thymine-requiring derivative of *E. coli* strain 15 during the thymineless state. These observations suggest that another enzyme, possibly a specific deoxyribose phosphate phosphatase, may operate in the deoxyribonucleoside and deoxyribonucleotide degradation pathway. It is not unreasonable that such an enzyme would be part of the *deo* operon and would be inducible by dR-5-P. Normally, in *deoC*$^+$ cells the dR-5-P aldolase is capable of degrading dR-5-P derived from added TdR, and no deoxyribose is excreted (5, 18). It is not known whether *deoB*$^-$ cells excrete deoxyribose.

It seems appropriate to speculate about the possible role of dR-5-P in the control of respiration and perhaps of DNA synthesis. We consider that the pathway: deoxyribonucleotide $\rightarrow$ deoxyribonucleoside $\rightleftarrows$ free base + dR-1-P $\rightleftarrows$ dR-5P $\rightarrow$ glyceraldehyde-3-P + acetaldehyde represents the route of degradation of deoxyribonucleotides and deoxyribonucleosides. Normally, dR-1-P and dR-5-P probably do not accumulate in quantity because of the excess of mutase and aldolase activities. Any block in the conversion of dUMP to dTMP or of the utilization of dTMP or of other deoxyribonucleotides for DNA synthesis would be expected to cause an accumulation of

deoxyribonucleotides and an increased formation of dR-5-P. Thus far, there is no evidence that conditions created by a block of dR-5-P aldolase or of dTMP synthetase will inhibit growth through an affect on respiration except by addition of deoxyribonucleosides to the media. It is possible that the affect is more subtle and might require an examination of synchronized cells. If such a control were to be operative, it could represent a mechanism for synchronizing DNA synthesis with energy need.

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