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# Mutants of *Salmonella typhimurium* Requiring Cytidine for Growth

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One would expect that the mutational loss of cytidine triphosphate synthetase (Fig. 1) reaction (9) would result in a strain which requires cytosine or cytidine for growth; however, to our knowledge, no such mutants have been isolated (B. Magasanik, p. 295-334, *In* I. C. Gunsalus and R. Y. Stanier [ed.], *The Bacteria*, vol. 3. Academic Press, Inc., New York, 1962). Failure to isolate mutants with specific requirements for cytosine or cytidine could be the result of: (i) the enzymatic inability of enteric bacteria to convert cytosine or cytidine to cytidine triphosphate, or (ii) the metabolic instability of C and CR because of their conversion by specific and highly active deaminases to uracil and uridine, respectively (Fig. 1, reactions 1 and 2). We have been successful in isolating mutants with specific requirements for cytidine from two strains in which reactions that degrade cytidine have been blocked by mutation. In one of these strains (DP-39), pyrimidine nucleoside phosphorylase (reactions 6 and 3) and cytidine deaminase (reaction 2) were blocked, while in the other strain (DL-38) both cytidine deaminase (reaction 2) and cytosine deaminase (reaction 1) were blocked.

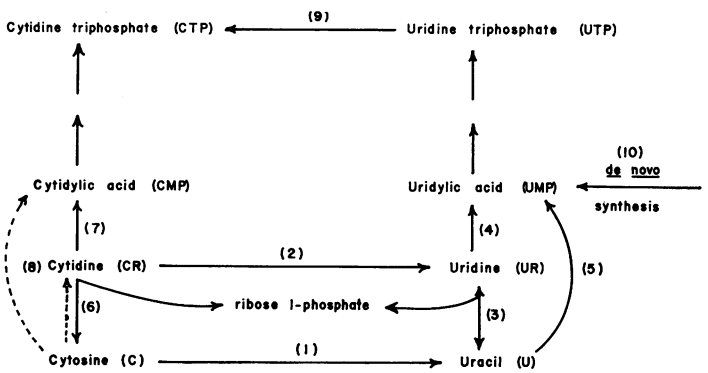


FIG. 1. *Interconversion of pyrimidines and their derivatives in Salmonella typhimurium. Solid arrows indicate reactions which have been shown to occur; broken lines indicate reactions which do not occur.*

Strain DP-39 was constructed in two steps from a pyr A mutant (deletion in the carbamyl phosphate synthetase structural gene) of *Salmonella typhimurium* LT-2 (Fig. 2). First, a mutant was isolated (DP-9) which lacked pyrimidine nucleoside phosphorylase activity (reaction 6 and 3). This was done by treatment with the mutagen nitrosoguanidine, followed by phenotypic expression in a glycerol minimal medium containing 50 $\mu$g of arginine per ml and 10 $\mu$g of uracil per ml, and by penicillin counterselection in the same medium with uridine serving as the sole source of carbon. DP-9 had lost the ability to utilize uridine or cytidine as a source of carbon (Table 1). From DP-9, a strain resistant to 5-fluorodeoxycytidine (FCdR) but still sensitive to 5-fluordeoxyuridine (FUdR) was isolated. This latter strain (DP-39) presumably lacked cytidine deaminase (reaction 2; O. Karlström, *unpublished data*) in addition to pyrimidine nucleoside phosphorylase and was unable to utilize cytidine either as a pyrimidine or as a carbon source.

Strain DL-38 was constructed in two steps from *S. typhimurium* LT-2 (Fig. 2). First, a strain (DL-25) resistant to FCdR and sensitive to FUdR was isolated and, from it, a strain (DL-38) resistant to 5-fluorcytosine but still sensitive to

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5-fluorouracil was isolated. This strain was presumed to have lost both cytidine- and cytosine-deaminases (reactions 1 and 2).

Mutants specifically requiring cytidine for growth (DP-55 and DP-45) were isolated from DP-39 and DL-38, respectively, by mutagenizing with nitrosoguanidine, phenotypically expressing in a minimal medium containing 50 μg/ml of both cytosine and cytidine, and counterselecting in the same medium lacking both cytosine and cytidine. The resulting mutants required cytidine for growth; neither cytosine nor deoxycytidine could replace cytidine. The cytidine triphosphate synthetase (reaction 9) assay of Long and Pardee (J. Biol. Chem. **242**:4715–4721, 1967) revealed activity in a parent strain (DL-38) but failed to demonstrate any detectable activity in either of the cytidine-requiring strains.

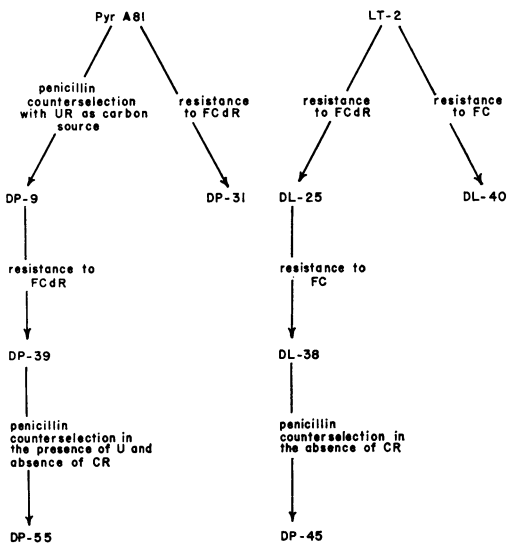


FIG. 2. *Scheme for the isolation of cytidine-requiring mutants.*

Availability of mutants genetically blocked between uridine triphosphate and cytidine triphosphate (reaction 9) provided a means of distinguishing between cytosine and uracil compounds as possible corepressors of the enzymes of pyrimidine biosynthesis. Strain DP-45, when deprived of cytidine, was shown to spill compound(s), equivalent to 5 μg of uracil per ml, into the medium which permitted the growth of a pyrimidine auxotroph (pyr A81), while the parent strain (DL-38), under the same conditions, spilled less than the equivalent of 1 μg/ml. Deprivation of a source of either uridine triphosphate or cytidine triphosphate results in a derepression of pyrimidine biosynthetic enzymes. The specific activity of aspartic transcarbamylase in DP-55 was 17-fold higher in cells grown in a culture limited by uracil content and 6-fold higher in cells grown in a culture limited by cytidine, as compared with a culture limited by glucose. Similarly, aspartic transcarbamylase activity of DP-45 was fivefold higher in cells from a cytidine-limited culture than in cells from a glucose-limited culture.

The fact that specific cytidine-requiring mutants have been isolated indicates that *S. typhimurium* possesses a functional cytidine kinase (reaction

TABLE 1. *Phenotypes of strains constructed to serve as parents for cytidine-requiring mutants*

| Strain designation | Presumed enzymatic deficiency | Compounds[a] which serve as | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pyrimidine source | | | | | Carbon source | | Analogue sensitivity | | | |
| | | U | UR | C | CR | CdR | UR | CR | FC | FU | FCdR | FUdR |
| Pyr A81 | (10) | + | + | + | + | + | + | + | s[b] | s | s | s |
| DP-9 | (10) (3) (6) | + | + | + | + | + | − | − | s | s | s | s |
| DP-31[c] | (10) (2) | + | + | + | + | − | + | − | s | s | r | r |
| DP-39 | (10) (3) (6) (2) | + | + | + | − | − | − | − | s | s | r | s |
| LT-2 | | 0[d] | 0 | 0 | 0 | 0 | + | + | s | s | s | s |
| DL-25 | (2) | 0 | 0 | 0 | 0 | 0 | + | − | s | s | r | s |
| DL-40[c] | (1) | | | | | | + | + | r | s | s | s |
| DL-38 | (2) (1) | | | | | | + | − | r | s | r | s |

[a] Abbreviations: U, uracil; UR, uridine; C, cytosine; CR, cytidine; CdR, deoxycytidine; FC, 5-fluorocytosine; FU, fluorouracil; FCdR, 5-fluorodeoxycytidine; FUdR, 5-fluordeoxyuridine.

[b] Indicates sensitivity to the drug; r indicates resistance.

[c] Not used in the present study. Included for comparison.

[d] No requirements.

7). The fact that cytosine cannot support the growth of this mutant indicates that no cytidine monophosphate pyrophosphorylase (reaction 8) is present, and that the pyrimidine nucleoside phosphorylase (reaction 6) is unable to convert cytosine into cytidine, in vivo.

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