JOURNAL OF BACTERIOLOGY, Oct. 1976, p. 242-247
Copyright © 1976 American Society for Microbiology
Vol. 128, No. 1
Printed in U.S.A.

Transport of Vitamin B_{12} in *tonB* Mutants of *Escherichia coli*

PHILIP J. BASSFORD, JR., CLIVE BRADBEER, ROBERT J. KADNER,* AND CARL A. SCHNAITMAN

Departments of Biochemistry and Microbiology, The University of Virginia School of Medicine, Charlottesville, Virginia 22901*

Received for publication 14 July 1976

It is known that the *tonB* mutation in *Escherichia coli* is responsible for a defect in the transport of iron chelates. These are transported by systems that involve outer membrane components. We found that *tonB* mutants were also deficient in the secondary, energy-dependent phase of vitamin B_{12} transport, although the mutants have normal levels of B_{12} receptors on their cell surface. In addition, *tonB* mutants derived from vitamin B_{12} auxotrophs required elevated levels of B_{12} for normal growth. Maltose uptake, mediated by another transport system involving an outer membrane component, was unaffected by the *tonB* mutation.

Receptor proteins in the outer membrane of cells of *Escherichia coli* and *Salmonella typhimurium* are intimately involved in the uptake of vitamin B_{12} and iron chelates. The *bfe* gene product of *E. coli* is an outer membrane protein of 60,000 molecular weight which is obligatory for the cellular adsorption of phage BF23, the three E colicins, and B_{12} (2, 6, 14, 15). The energy-independent binding of B_{12} to the *bfe* product has been shown to be the first step in the transport of this vitamin by *E. coli*. Numerous mutations that affect the uptake of iron chelates are known. According to the available evidence, the *tonA* product is an 85,000-molecular-weight protein that binds phages T1, T5, and ϕ80, colicin M, and iron-ferrichrome (1, 11, 12, 23). A separate protein, which may be the product of the *feu* locus, appears to function as the initial receptor for colicins B and D and for the ferric-enterochelin complex (13, 24). Finally, the uptake of maltose and maltodextrins is facilitated by the function of the product of the *lamB* gene, the phage lambda receptor (18).

Mutations in the *tonB* locus of *E. coli* confer resistance to bacteriophages T1 and ϕ80 and all of the group B colicins (3). In addition, *tonB* mutants are chromium sensitive (22), resistant to the siderophore antibiotic albomycin (12), hyperexcrete enterochelin (9), and are totally deficient in the uptake of all iron chelates. However, these mutants are still capable of reversibly binding phages T1 and ϕ80, and they retain receptor activity for the colicins and iron chelates (3, 11, 16). These observations, together with the finding that no outer membrane proteins appear to be missing in *tonB* mutants (3), suggest that the *tonB* product is involved in some step of colicin or ferric-enterochelin uptake subsequent to binding to the receptor.

The activities of the uptake systems for a number of amino acids and inorganic phosphate were normal in *tonB* mutants (8). However, none of these transport systems appears to employ components in the outer membrane. Hence, the effect of alterations in *tonB* on the function of other transport systems utilizing outer membrane components was investigated. This paper will demonstrate that *tonB* mutants are totally deficient in the energy-dependent uptake phase of vitamin B_{12} transport but are unaffected in maltose transport, even at low external concentrations of the substrate.

MATERIALS AND METHODS

Bacterial strains and media. The strains of *E. coli* K-12 employed in this study are listed in Table 1. Selection for phage- or colicin-resistant mutants was as described (15). Mutants in *tonB* were selected for simultaneous resistance to phage ϕ80*vir* and colicin D. A small percentage of these (less than 5%) simultaneously acquired a tryptophan auxotrophy; these are designated as Δ*tonB-trp*. All presumptive *tonB* mutants were tested for their sensitivity to phages T5 and BF23. Mutants in *tonA* were characterized as those resistant to phages T5 and ϕ80*vir* but sensitive to phage BF23 and colicin D. A number of colicin D-insensitive mutants that were sensitive to phages T5, ϕ80*vir*, and BF23 were isolated and tested.

Mutants in *lamB* were obtained as those resistant to phage λcI which were still Mal^+ on MacConkey maltose plates (19). Cloned isolates were tested for their resistance to phage λcI and their sensitivity to phages λh80 (to eliminate possible λ lysogens) and BF23.

Minimal salts medium A (4) was supplemented with glucose at 0.5%, required amino acids (100 μg/

TABLE 1. *List of bacterial strains and phage stocks employed*

Strain	Genotype	Source
RK4126	*proC lysA metE argH strA*	RK4101 (15)
1485F⁻	Wild-type K-12	B. Bachman, and cured of F with acridine orange.
RK4129	as RK4126, but *tonB15*	
RK4128	as RK4126, but *tonB2*	
RK4127	as RK4126, but *bfe*	
RK4130	as RK4127, but *tonB2*	
CA23	Colicinogenic for colicin D	T. Pugsley
RK1044	*entA ilv nalA*	AN248 from G. Cox
Phage stocks		
BF23		R. Benzinger
φ80vir		J. R. Johnson
λcI (W30)		M. Gottesman
λh80 (W248)		M. Gottesman
T5		Laboratory stock

ml), and thiamine (1 μg/ml). Strains were induced for maltose transport by growth with 0.4% maltose as carbon source.

Transport assays. The uptake of cyanocobalamin (B_{12}) was assayed by the procedure described by DiGirolamo and Bradbeer (5). Cells grown at 37°C were harvested in the exponential phase, washed with medium A or 0.1 M potassium phosphate, pH 6.6. Washed cells at ca. 5×10^8/ml were incubated in the presence of glucose with [^{3}H]cyanocobalamin. At intervals, portions were withdrawn, passed through membrane filters (Millipore Corp., 0.45-μm pore size) and washed with 5 ml of buffer. Correction was made for the binding or trapping of substrate by the filter in the absence of cells. Measurement of B_{12} binding to whole cells employed cells incubated for 10 min with dinitrophenol (5 mM) (5).

The assay for maltose transport was similar to the fast assay described by Szmelcman and Hofnung (18), except that the buffer for washing and suspension of the cells was medium A. Induced cells were washed once in medium A and suspended in medium A containing chloramphenicol (40 μg/ml) for 15 min before assay. After addition of [^{14}C]maltose (7.9 Ci/mol) to final concentrations of 3.5 or 35 μM, portions were filtered after 30, 60, 90, and 120 s, as described above. Filters were washed with 5 ml of medium A, air-dried, and counted in a scintillation counter with toluene-based fluor.

Chemicals. Colicin D was prepared following growth of the colicinogenic strain (CA 23) in L broth to a density of 4×10^8/ml. The culture was induced by exposure to 0.2 μg of mitomycin C per ml and incubated at 37°C for 90 min, after which chloroform was added. The culture supernatant solution was clarified by centrifugation and stored at 4°C, at which temperature it was stable for several weeks.

Radioactively labeled amino acids were obtained from New England Nuclear Corp. Labeled maltose and cyanocobalamin were from Amersham/Searle Co. Other chemicals were from Sigma Chemical Co.

RESULTS

B_{12} uptake in *tonB* mutants. Mutants altered at *tonB* are defective in the uptake of ferric chelates (8, 21, 22). For the sake of comparison, the uptake of B_{12} was measured in several independent *tonB* mutants derived from each of three parental strains. This collection of mutants included those that had acquired a tryptophan auxotrophy, associated with a deletion from *tonB* at least through to the nearby *trp* locus. All of the *tonB* mutants tested were totally deficient in the secondary, concentrative phase of B_{12} uptake (Fig. 1). The data for only two unrelated *tonB* mutants and their parental strains are shown, but identical results were obtained for 18 additional mutants, including some point mutants, as detailed below. The initial phase of uptake, corresponding to binding to the outer membrane receptor, was measured in cells treated with the energy poison, dinitrophenol. The level of B_{12} binding by whole cells was essentially unaltered in all of the *tonB* mutants relative to that in the parental strains (Table 2). These results are consistent with the full sensitivity of *tonB* mutants to phage BF23 and the E colicins which share the B_{12} receptor.

Frost and Rosenberg (8) showed that *tonB* mutants are not altered in other nutrient transport systems not employing outer membrane constituents, such as arginine, serine, proline,

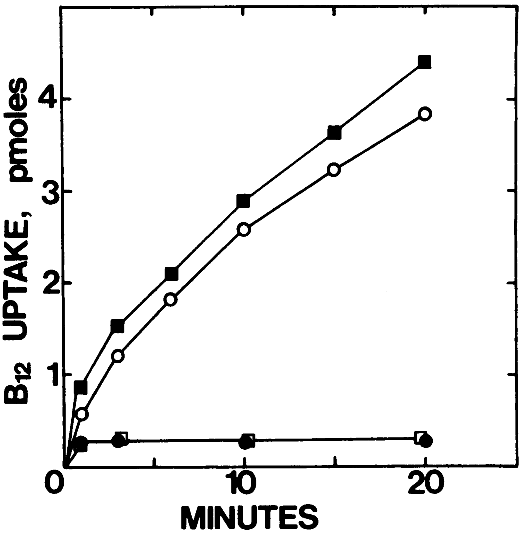

FIG. 1. *Uptake of B_{12} in tonB mutants. The strains used were RK4126 (○), RK4128 (●), 1485F⁻ (■), and 1485F⁻ ΔtonB-trp (□). Cells were grown in minimal growth medium to mid-logarithmic phase and assayed as described in Materials and Methods. Samples are expressed as pmol of B_{12} taken up per 10^9 cells. Cells were counted with a Petroff-Hauser slide.*

TABLE 2. B_{12} *binding by dinitrophenol-treated tonB mutants*

Strain	Relevant genotype	B_{12} bound[a] (pmol/10^9 cells)	Receptors/cell[b]
RK4126	*ton*$^+$	0.26	158
RK4128	*tonB*2	0.22	134
RK4129	*tonB*15	0.19	115
1485F$^-$	*ton*$^+$	0.35	211
1485F$^-$	Δ*tonB-trp*	0.21	127

[a] External B_{12} concentration was 8.6 nM.
[b] As determined from B_{12} binding.

and inorganic phosphate. The uptake of glutamine, histidine, and proline was normal in the four *tonB* mutants examined by us. Leucine uptake was also normal except for that in a strain carrying a Δ*tonB-trp* deletion. This strain had only 20% of the wild-type level of leucine transport. Thorne and Corwin (20) had shown that analogous deletions in *S. typhimurium* were defective in leucine uptake.

The possibility existed that the defect observed in vitamin B_{12} uptake in *tonB* mutants did not result from a defective *tonB* gene product, but rather resulted from the high levels of enterochelin known to be excreted into the culture medium by *tonB* mutants (9). It seemed unlikely that enterochelin was competing with B_{12} for a component of the specific B_{12} transport system, since both these substrates utilize different receptors on the cell surface. Two experiments were done which eliminated this possibility. First, it was shown that vitamin B_{12} transport was not restored in *tonB* mutants by growth in the presence of 100 μM $FeSO_4$, which is more than sufficient to repress enterochelin biosynthesis, even in a *tonB* mutant (21). Second, *tonB* mutants were isolated in a strain (RK1044) that carried an *entA* mutation. Young et al. (25) had shown that *entA* mutants were specifically blocked in the pathway leading to the biosynthesis of enterochelin. When B_{12} transport was examined in *entA tonB* double mutants, the secondary phase of B_{12} transport was still not detectable.

Other mutations affecting iron uptake did not affect B_{12} uptake. Mutations in *tonA*, which abolish cellular receptor activity for iron-ferrichrome (23), had no effect on the uptake or binding of vitamin B_{12} in nine independent mutants tested. Similarly, four colicin D-insensitive mutants, which were still sensitive to all of the phages tested, had normal B_{12} transport. Thus, the elimination of the secondary phase of B_{12} uptake appears to be a result of the *tonB* mutation.

Growth on B_{12} by *tonB* mutants. Since *tonB* mutants are completely devoid of B_{12} uptake by the usual assay, it seemed likely that they would be unable to utilize B_{12} as a growth-limiting nutrient. Hence, *tonB* mutants were selected in *metE* strains, which require either methionine or B_{12} for growth (4). The *tonB*$^+$ *metE* parental strain (RK4126) responds well on plates to B_{12} concentrations as low as 5×10^{-10} M, with partial growth at 5×10^{-11} M. A *bfe* derivative, RK4127, grows well only at 5×10^{-6} M B_{12} and poorly at 5×10^{-7} M (Table 3).

An examination of the ability of over 300 *tonB* mutants to grow on 5×10^{-9} M B_{12} revealed the existence of two types of responses. The major class, comprising about 75% of the mutants and including all of the Δ*ton-trp* deletion strains, gave no growth on this level of B_{12}. The minority class was capable of nearly normal growth under these conditions. Members of the majority class, characterized by strain RK4128, could grow well on plates containing 5×10^{-7} M B_{12}. The introduction of a *bfe* mutation to this strain, yielding strain RK4130, further reduced the response to B_{12} to the level seen in the *ton*$^+$ *bfe* strain RK4127. Members of the minority class, such as strain RK4129, were only partially impaired in their response to B_{12}.

Despite these differences in growth response, there was no detectable difference in B_{12} uptake properties of the strains tested in the usual transport assay. There was no secondary-phase activity detected in either class of mutant. As before, the extent of B_{12} binding in energy-poisoned cells was normal in all strains tested. This result is in keeping with other observations that the ability of cells to utilize B_{12} for growth is a more sensitive assay of its uptake than is the usual transport assay with isotopically labeled substrate (15). However, even by this more sensitive criterion, the *tonB* product is clearly implicated in the uptake of B_{12}.

B_{12}-utilizing revertants from *tonB* mutants. Further information on the relationship of *tonB* to B_{12} uptake was obtained from the isolation of B_{12}-utilizing revertants of many of the *tonB* strains. Revertants from the major class of *tonB* mutants, which were unable to respond to 5×10^{-9} M B_{12}, were selected on 5×10^{-10} M B_{12}. Two classes of revertants could be discerned. One type was generated by all the *tonB* mutants tested, including *tonB-trp* deletions, and were recovered at high frequencies ranging from 10^{-4} to 10^{-5} per cell plated. These form small, mucoid colonies on 5×10^{-10} M B_{12} and are still resistant to colicin D and phage φ80*vir*. In addition, they are quite unstable, such that growth of cloned isolates on L plates or minimal medium with methionine instead of

TABLE 3. *Growth of tonB mutants with B_{12} as methionine source*

Strain	Relevant genotype	Response on minimal agar plates with[a]						
		Methionine (100 μg/ml)	B_{12} (M)					
			5×10^{-6}	5×10^{-7}	5×10^{-8}	5×10^{-9}	5×10^{-10}	5×10^{-11}
RK4126	*metE*	3+	3+	3+	3+	3+	3+	+
RK4127	*metE bfe*	3+	3+	+				
RK4128	*metE tonB2*[b]	3+	3+	3+	+			
RK4129	*metE tonB15*[c]	3+	3+	3+	3+	2+	+	
RK4130	*metE tonB2 bfe*	3+	3+	+				

[a] Determined by relative colony size compared with strain RK4126 on methionine after 48 h of growth on minimal growth medium with the indicated supplements.

[b] This strain is a representative of the major class of *tonB* mutant; it appears to be a point mutation, but deletion strains behave identically.

[c] This is a representative of the minor class of *tonB* mutants.

B_{12} results in a rapid loss of the ability to utilize B_{12}. These strains are similar to a class of revertants of *bfe* strains that will be discussed in a future communication. In essence, these result from a secondary lesion producing an alteration in the outer membrane increasing its permeability for B_{12}, thereby bypassing the usual receptor-mediated route of entry.

A second class of revertants was derived from only a few *tonB* mutants. This class formed large colonies on 5×10^{-10} M B_{12}; members of this group were sensitive to colicin D and phage $\phi80vir$ and exhibited normal, wild-type levels of B_{12} binding and uptake. These are presumably revertants to $TonB^+$. These are not generated by any of the $\Delta tonB$-*trp* strains or by a number of Trp^+ strains, which may have been generated by short deletions. Presumably, point mutants in *tonB* are able to give rise to this class of true revertants. This confirms that it is the loss of the *tonB* product alone that is responsible for the defect in B_{12} uptake, rather than the loss by deletion of some adjacent locus.

Revertants of the minor class of *tonB* mutants that were able to grow on moderately low levels of B_{12} were difficult to obtain and characterize. These mutants presumably represent missense mutations resulting in the presence of an altered but still partially functional *tonB* gene product. In spot tests, these mutants are still slightly sensitive to phage $\phi80vir$, although not to colicin D. All of these results support the conclusion that the *tonB* product must be functional to allow the uptake of B_{12} or ferric chelates.

Maltose transport in *tonB* mutants. Szmelcman and Hofnung (18) have shown that the product of the *lamB* gene, the phage λ receptor, participates in the uptake of maltose at low concentrations. The basic piece of evidence for this, which we have confirmed, was that some *lamB* mutants exhibited greatly decreased rates of uptake of maltose at low external concentrations (3.5 μM) relative to the parental strain. Uptake in the mutant at higher external concentrations approached that of the parent strain. Missense mutants in *lamB* were less severely affected in maltose uptake than were nonsense or deletion mutants. The uptake of 3.5 μM maltose was measured over a 2-min time period in ton^+ and *tonB* derivatives of lam^+ and *lamB* strains. There was no significant effect of the state of the *ton* locus on maltose uptake at either 3.5 or 35 μM maltose. Several of the *tonB* mutants studied here carried a $\Delta tonB$-*trp* deletion, and all were totally deficient in vitamin B_{12} uptake. Thus, the *tonB* product, although required for uptake of B_{12} or ferric chelates, is apparently not involved in maltose uptake, even under conditions where that uptake is dependent on an outer membrane component.

DISCUSSION

Several studies have implicated the *tonB* product as being a common component of the various iron chelate transport systems (8, 21, 22), based primarily on the loss of all measured iron chelate transport activities. The *tonB* product would appear not to be the initial receptor for the iron chelates for several reasons. First, distinct mutants have been found which appear to be defective in individual iron chelate uptake systems, such as that for ferric-ferrichrome (12, 23) and for ferric-enterochelin (13). In fact, the *tonA* product has been strongly implicated as being the cell surface receptor for ferric-ferrichrome. Secondly, mutants in *tonB* retain receptor activity for phages T1, T5, and $\phi80$ (11), all the group B colicins, and for ferric-enterochelin (3, 16).

However, from the results presented in this paper, it is clear that the *tonB* product is also required for the uptake of vitamin B_{12}, which has never before been shown to be related to any of the iron uptake systems. Along this line, neither phage BF23 nor the three E colicins that share the B_{12} receptor are affected by the *tonB* mutation.

Explanations for these observations may well be expressed in terms of the architecture of the outer membrane and, possibly, in specialized regions of communication of the outer membrane receptors with the putative transport systems in the cytoplasmic membrane. Results of Haller and colleagues (10) and Rosenbusch (17) are suggestive that outer membrane proteins are arranged in complex asymmetric arrays. It is not inconsistant with the available evidence that the *tonB* product could be involved in maintenance of the proper orientation of receptors in the outer membrane with their sites for subsequent uptake. It would not be unexpected that transport systems totally dependent on the function of an outer membrane receptor might have very strict requirements for the proper orientation of its components between the two membranes.

The failure of the *tonB* mutation to adversely affect maltose uptake is, in fact, consistent with this hypothesis. On the basis of the current evidence, the maltose uptake system appears to be quite distinct from that for B_{12} and the iron chelates. Unlike the case for vitamin B_{12} or the iron chelates, there is no detectable binding of maltose or any higher maltodextrin to the phage λ receptor, either in vivo or in vitro (18a). Furthermore, maltose uptake is only dependent on the function of the *lamB* product at very low external concentrations (18). Even under these conditions, the uptake of maltose is absolutely dependent on its binding to the maltose-binding protein located in the periplasm. Thus, no specific orientation of the *lamB* product in the outer membrane with any component in the cytoplasmic membrane need be invoked, since the substrate must appear in the periplasm before uptake. Although the genetics of B_{12} or iron uptake are not as advanced as that for the maltose pathway, there is so far no evidence in the former two systems for the obligate involvement of periplasmic proteins.

Experiments are now in progress to attempt to identify the nature and function of the *tonB* product, information on which has not yet been obtained.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants GM19078 (R.J.K.), from the National Institute of General Medical Sciences, and AM12653 (C.B.), from the National Institute of Arthritis, Metabolism, and Digestive Diseases, and by grant BMS-04973 (C.A.S.) from the National Science Foundation. R.J.K. is the recipient of Research Career Development award GM00019 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Braun, V., K. Schaller, and H. Wolff.** 1973. A common receptor protein for phage T5 and colicin M in the outer membrane of *Escherichia coli* B. Biochim. Biophys. Acta 323:87-97.
2. **Buxton, R. S.** 1971. Genetic analysis of *Escherichia coli* K12 mutants resistant to bacteriophage BF23 and the E-group colicins. Mol. Gen. Genet. 113:154-156.
3. **Davies, J. K., and P. Reeves.** 1975. Genetics of resistance to colicins in *Escherichia coli* K-12: cross-resistance among colicins of group B. J. Bacteriol. 123:96-101.
4. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. 60:17-28.
5. **DiGirolamo, P. M., and C. Bradbeer.** 1971. Transport of vitamin B_{12} in *Escherichia coli*. J. Bacteriol. 106:745-750.
6. **DiMasi, D. R., J. C. White, C. A. Schnaitman, and C. Bradbeer.** 1973. Transport of vitamin B_{12} in *Escherichia coli*: common receptor sites for vitamin B_{12} and the E colicins on the outer membrane of the cell envelope. J. Bacteriol. 115:514-521.
7. **Frost, G. E., and H. Rosenberg.** 1973. The inducible citrate-dependent iron transport system in *Escherichia coli* K12. Biochim. Biophys. Acta 330:90-101.
8. **Frost, G. E., and H. Rosenberg.** 1975. Relationship between the *tonB* locus and iron transport in *Escherichia coli*. J. Bacteriol. 124:704-712.
9. **Guterman, S. K., and L. Dann.** 1973. Excretion of enterochelin by *exbA* and *exbB* mutants of *Escherichia coli*. J. Bacteriol. 114:1225-1230.
10. **Haller, I., B. Hoehn, and U. Henning.** 1975. Apparent high degree of asymmetry of protein arrangement in the *Escherichia coli* outer cell envelope membrane. Biochemistry 14:478-484.
11. **Hancock, R. E. W., and V. Braun.** 1976. Nature of the energy requirement for the irreversible adsorption of bacteriophages T1 and ϕ80 to *Escherichia coli*. J. Bacteriol. 125:409-415.
12. **Hantke, K., and V. Braun.** 1975. Membrane receptor dependent iron transport in *Escherichia coli*. FEBS Lett. 49:301-305.
13. **Hantke, K., and V. Braun.** 1975. A function common to iron-enterochelin transport and action of colicins B, I, V in *Escherichia coli*. FEBS Lett. 59:277-281.
14. **Jasper, P. E., E. Whitney, and S. Silver.** 1972. Genetic locus determining resistance to phage BF23 and colicins E_1, E_2 and E_3 in *Escherichia coli*. Genet. Res. 19:305-312.
15. **Kadner, R. J., and G. L. Liggins.** 1973. Transport of vitamin B_{12} in *Escherichia coli*: genetic studies. J. Bacteriol. 115:514-521.
16. **Pugsley, A. P., and P. Reeves.** 1976. Iron uptake in colicin B-resistant mutants of *Escherichia coli* K-12. J. Bacteriol. 126:1052-1062.
17. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein from *Escherichia coli*. J. Biol. Chem. 249:8019-8029.
18. **Szmelcman, S., and M. Hofnung.** 1975. Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. 124:112-118.

18a. **Szmelcman, S., M. Schwartz, T. J. Silhavy, and W. Boos.** 1976. Maltose transport in *Escherichia coli*: a comparison of transport kinetics in wild-type and

resistant mutants with the dissociation constants of the maltose binding protein as measured by fluorescence quenching. Eur. J. Biochem. 65:13-19.
19. **Thirion, J. P., and M. Hofnung.** 1972. On some genetic aspects of phage λ resistance in *E. coli* K-12. Genetics 71:207-216.
20. **Thorne, G. M., and L. M. Corwin.** 1972. Genetic locus of a gene affecting leucine transport in *Salmonella typhimurium*. J. Bacteriol. 110:784-785.
21. **Wang, C. C., and A. Newton.** 1969. Iron transport in *Escherichia coli*: roles of energy-dependent uptake and 2,3-dihydroxybenzoylserine. J. Bacteriol. 98:1142-1150.
22. **Wang, C. C., and A. Newton.** 1971. An additional step in the transport of iron defined by the *tonB* locus of *Escherichia coli*. J. Biol. Chem. 246:2147-2151.
23. **Wayne, R., and J. B. Neilands.** 1975. Evidence for common binding sites for ferrichrome compounds and bacteriophage φ80 in the cell envelope of *Escherichia coli*. J. Bacteriol. 121:497-503.
24. **Wayne, R., K. Frick, and J. B. Neilands.** 1976. Siderophore protection against colicins M, B, V and Ia in *Escherichia coli*. J. Bacteriol. 126:7-12.
25. **Young, I. G., L. Langman, R. K. J. Luke, and F. Gibson.** 1971. Biosynthesis of the iron-transport compound enterochelin: mutants of *Escherichia coli* unable to synthesize 2,3-dihydroxybenzoate. J. Bacteriol. 106:51-57.